JOURNAL OF DAIRY SCIENCE

NOVEMBER, 1956

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The Control of Rancidity in Milk

Gestation Length for Holstein-Friesians

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Letters to the Editor

Student Chapter News

Technical Notes

Important News and Literature Reviews

Six Manufacturing and Seven Production Research Articles

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, P. H. Tracy, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

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Advertising copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the editor, P. H. Tracy, at the above address. Advertising plates should be sent directly to The Garrard Press, 119-123 West Park Ave., Champaign, Ill.

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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

J. M. Sherman Dies

Word of the death on November 5 of DR. J. M. SHERMAN, former head of the Department of Dairy Industry at Cornell University, has been received. Further details will be published in the December issue.

Pioneers in the Dairy Industry

Outstanding as a researcher, teacher, scholar, administrator, inventor, and friend is the man, DR. B. W. HAMMER. Modest of his many accomplishments, Dr. Hammer is a person reluctant to be placed in the spotlight of fame, a quality which adds to his stature.

Bernard Wernick Hammer was born October 7, 1886, in Hillsboro, Wisconsin. In 1908

Dr. and Mrs. B. W. Hammer

he obtained a B.S.A. degree from the University of Wisconsin. A year later he was married to Edna Quamman of Madison. Their only child, Robert Q., was born in Ames, Iowa, in 1913.

After completing his undergraduate work, young Hammer continued his studies while serving as an assistant for one year in agricultural bacteriology under DR. E. G. HASTINGS at the University of Wisconsin. From 1909 to 1911 he was associated with DR. M. P. RAVENEL at the Wisconsin State Hygienic Laboratory. He then joined the Iowa State College and in 1916 became chief in dairy bacteriology in the Agricultural Experiment Station.

Graduate study and research were continued and in December of 1920 he was awarded the Ph.D. degree by the University of Chicago. Research in dairy bacteriology at Iowa State then began in earnest as evidenced by the parade of students at both the graduate and undergraduate level through the classes of Dr. Hammer. In 1942 he resigned his position at Iowa State to become a member of the research staff of Golden State Co., Ltd., San Francisco.

In 1946 Dr. Hammer retired to live at Sarasota, Florida, where he now divides his time between his hobbies and consulting work. Both Dr. and Mrs. Hammer are ardent golfers and gardeners. Fishing also comes in for its share of attention. Dr. Hammer makes frequent business trips to the North but when the fish are running he is likely to be found on location in Florida.

Dr. Hammer is the author or coauthor of many scientific articles in the field of dairy bacteriology. His book *Dairy Bacteriology*, which has undergone numerous revisions since its first publication in 1928, has been widely used. That Dr. Hammer is an inventor as well as researcher and teacher is evidenced by the numerous patents that bear his name, one of the most important of which has to do with the process of continuous buttermaking. The many contributions that have been made during the lifetime of B. W. Hammer to the progress of dairying throughout the world have justly won for him the recognition of being one of our great "Pioneers of the Dairy Industry."

A.O.A.C. to Give Annual Award

KENNETH D. JACOB, president of the Assoc. of Official Agricultural Chemists, has announced the establishment of the Harvey W. Wiley Award for analytical methods. The award honors the memory of the founder of the federal Pure Food and Drug Law, who was also one of the founders of the Association.

Mr. Jacob stated that the annual award, which carries a cash prize of $500, will be presented to the scientist or group of scientists who make an outstanding contribution to development of methods of analysis of foods, cosmetics, drugs, feeds, fertilizers, pesticides, and soil, as well as for methods in general analytical chemistry. These are the fields of interest of the A.O.A.C.,

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encompassed by its publication, the *Official Methods of Analysis*. This 1,000-page book is the primary laboratory manual of regulatory chemists and research workers in agriculture in the U. S. and throughout the world.

The first award will be given at the 71st annual meeting of the Association in October, 1957. All scientists, whether members or nonmembers of the A.O.A.C., are eligible for the award.

New Editor of Journal Appointed

E. O. HERREID of the Dept. of Food Technology of the Univ. of Illinois has been selected by the official board to serve as editor-in-chief of the Journal to succeed P. H. TRACY, who tendered his resignation last June. Dr. Herreid takes over his official duties with the January 1957 issue.

Dr. Herreid, a veteran of World War I, took his undergraduate work at South Dakota Agricultural College and his graduate work at Minnesota. Before joining the Illinois staff he was a member of the Dairy Dept. at the Univ. of Vermont. He is the winner of the 1956 Borden Award for outstanding research in the field of dairy manufactures.

All manuscripts and communications pertaining to material to be published in the Journal after the December 1956 issue should be sent to the new editor. His address is 101 Dairy Manufactures Building, University of Illinois, Urbana, Ill.

New Section Formed by Ecological Society

At the recent meeting of the Ecological Society of America at Storrs, Conn., a Section of Animal Behavior and Sociobiology was formally organized. The purpose of this Section is to advance, coordinate, and assist research and publications on the subject of animal behavior and social organization basic to theoretical science and human welfare and to act as a liaison agency between workers in the various scientific fields concerned.

At its first organizational meeting, the Section elected the following officers: chairman, J. P. SCOTT, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine; vice-chairman, A. M. GUHL, Kansas State College, Manhattan; secretary, M. W. SCHEIN, Pennsylvania State Univ., University Park.

The Section immediately concerned itself with three major problems in the field of behavior studies: publications, terminology, and teaching. L. R. ARONSON, the American Museum of Natural History, was named as chairman of a committee to continue the search for new publication outlets for papers dealing with animal behavior. A. M. GUHL, Kansas State College, was appointed chairman of a Committee on Glossary and Terminology designed to help alleviate the confusion caused by coining new terms or misusing old ones. W. M. ATKIN, Albert Einstein College of Medicine, was named as chairman of a committee to look into the needs and possibilities of publishing a textbook on animal behavior and sociobiology aimed at the upper-class or early graduate student level.

A tentative list of members of the Section includes a wide variety of interests, covering every form of animal life as well as the more generalized biological fields. Persons interested in any phase of behavior studies are invited to become members. Any member of any class of the Ecological Society of America may become a member merely by writing to the secretary of the Section; there are no additional dues. Others may join the Section by becoming members of the Ecological Society and then informing the secretary of the Section of their action. Further information may be obtained from the secretary.

Michigan State Holds Dairy Conference

M.S.U.'s 17th Annual Dairy Manufacturers' Conference was held at the Kellogg Center Nov. 7 and 8. Besides a general meeting and a clinic, special sessions were held for market milk, ice cream, and manufactured products on each of the 2-day meetings. One afternoon was devoted to conducted tours of the new Animal Industries Building which houses, besides the Poultry and Animal Husbandry Departments, the Meat Products laboratory, the Dairy Department, and the Creamery.

The banquet was held Nov. 7, and a special luncheon, with a speaker, on each day of the session.

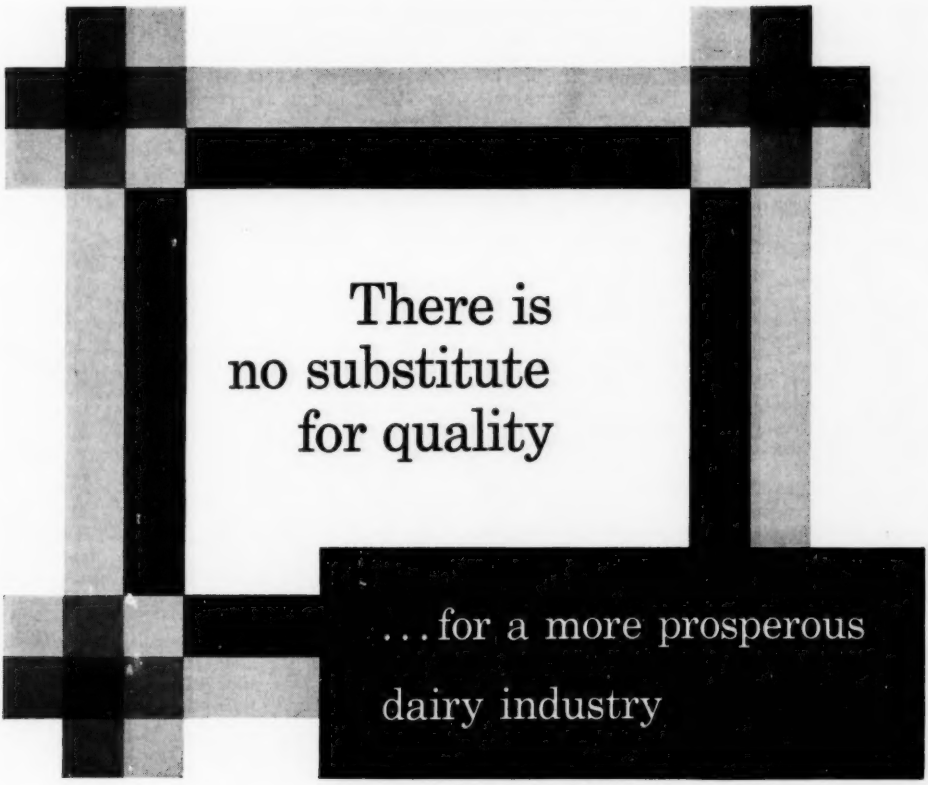
News Items from the University of Georgia

W. J. MILLER has returned after participating for 3 months in the Univ. of Tennessee–Atomic Energy Commission agricultural research program. Dr. Miller now is devoting full time to research and is in charge of the Dairy Cattle Nutrition Laboratory.

GERALD STOTT joined the Dairy Dept. staff on Sept. 1. His B.S. and M.S. degrees were earned at Utah State Agricultural College and his Ph.D. degree at the Univ. of Wisconsin. He has held teaching and research assistantships at both institutions. His new duties include primarily teaching of dairy production subjects, but some time will be devoted to research.

Six scholarships valued at $200 each have been assigned for upper-classmen in dairying during the fall quarter.

As soon as the plans come from the architests' drawing boards, the University will construct a $10,000,000 Science Center. Four fully equipped buildings will be included in



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the group. It is expected that contracts will be signed very soon for the construction of the Chemistry and Animal Science Buildings. The latter will house the Animal Husbandry, Dairy Production, and Poultry Departments. A recent allocation provides $5,000,000 for the housing of Biological Sciences and Physics. Buildings for Mathematics, Geography, Geology, and Food Technology will be constructed later to complete the Center.

Ohio State Honors Outstanding Dairyman

The Dept. of Dairy Technology of The Ohio State Univ. presented V. O. DREYER, manager of the Borden Company, Dayton, its Award of Merit at the Eighth Annual Homecoming Brunch, Oct. 27. The award, which has been presented annually since 1949, is for outstanding contributions to the Ohio dairy industry. Assistant Dean C. S. HUTCHISON of the College of Agriculture made the presentation.

The recipient has served the dairy industry of Ohio as president of the Ohio Milk Distributors Assoc., president of the Ohio Dairy Products Assoc., and as vice-president of the Ohio American Dairy Assoc. since 1947. He has been active in a number of local dairy and civic affairs.

Dairy Plant Fieldmen Meetings

Five Dairy Plant Fieldmen meetings will be held in Ohio during December, 1956. Production, quality, herd health, and farmer-fieldman relationships will be discussed. One of the main features of the program will be a discussion by DR. DAVE HILL of the State Veterinarians Office, on the new proposed brucellosis law for Ohio.

H. F. JUDKINS, secretary-treasurer of the A.D.S.A., was the featured speaker at the October meetings of the Maumee Valley, Northeastern Ohio, and Central Ohio Dairy Technology Societies. The title of Mr. Judkins' speech was "Efficiency and Dairy Plant Operations."

Illinois Wins First in International Dairy Products Judging Contest

The three-man team from the Univ. of Illinois, coached by Dr. Joseph Tobias, walked away with the top honors over 32 other colleges in the annual Olympics of dairy judging, Oct. 30, in Atlantic City. Fred Jaronitzky led the Illinois attack, winning for himself the highest individual score of any of the 99 individual contestants. The Illinois team received a graduate fellowship of $1,800 and possession of the All-Products Bowl at a banquet and awards ceremonies at the Chalfonte Hotel.

Second and third place all-products winners were the team from Iowa State College, which received a $1,650 fellowship, and the team from Mississippi State College, which received a $1,500 fellowship.

A comparative newcomer in the dairy produtes judging world, the Univ. of Maryland, took top honors in ice cream judging and gained possession of the Ice Cream Cup. The team from the Univ. of Wisconsin took top honors in milk judging and received the Milk Cup. The Butter Cup for excellence in butter judging went to the team from Ohio State. Cheese judging excellence brought the Cheese Cup to Iowa State College.

Individual winners in each of the five divisions of the Contest also received special awards. Fred Jaronitzky of Illinois, the highest scorer in the All Products Division, received a fine watch. Placing second in All Products was Don W. Gibson of California State Polytechnic Institute, who received a silver medal and gained additional honor for the California team by also winning a silver medal in the Milk Division. Third place and a bronze medal in All Products went to Charles M. Mead of Iowa State, who also won the silver medal in the Butter division.

In the Ice Cream division, top individual honors and a wrist watch went to Ronald Sappington of the Univ. of Maryland. Second place and a silver medal went to Wesley E. Eckert of Washington State College. Third place and a bronze medal were awarded to Jerome J. Donovan of the Univ. of Massachusetts.

In the Milk division, individual honors were as follows: first place and a wrist watch to Lyle Surprise of the Univ. of Wisconsin; second place and a silver medal to Don Gibson of California Polytechnic; third place and a bronze medal to William Breene of the Univ. of Wisconsin.

In the Butter division, individual honors were as follows: first place and a wrist watch to Fred Dryg of the Univ. of Minnesota; second place and silver medal to Charles Mead of Iowa State; third place and Bronze medal to Richard F. Flowers of Ohio State Univ.

In the Cheese division, individual honors were: first place and wrist watch to Thomas E. Quint, Kansas State College; second place and silver medal to Thomas Ruzicka of Iowa State; third place and bronze medal to Ronald Upshaw of Oklahoma A. & M. College.

Awards were presented to winners at a mock television show which followed the awards banquet. Master of ceremonies was comedian Roger Price, inventor of "Droodles," who announced winners as their faces were flashed on a mock television screen on a stage in the room. All of the 33 teams participating in the contest had furnished contest officials with pictures of the team prior to the contest, in order that slides might be prepared for every team.

More colleges than ever before competed in the collegiate contest this year — 33 from every section of the United States — and contest officials were impressed with the very fine showings made by the colleges who were entering

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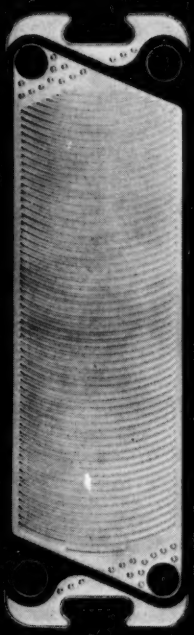

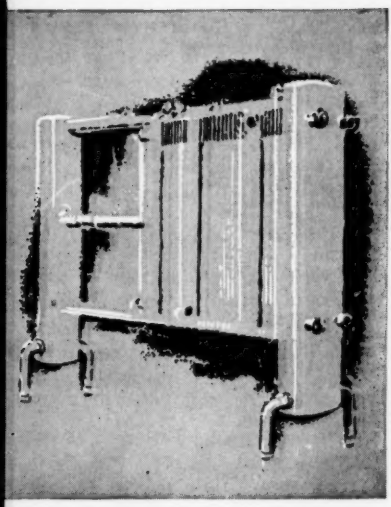

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the contest for the first time. Sponsored since 1930 by American Dairy Science Assoc. and Dairy Industries Supply Assoc., the contest is held regularly in conjunction with the autumn conventions of the major U.S. dairy industrial trade associations and with the Dairy Industries Exposition.

Following is a list of those who won high standings in the contest:

ALL PRODUCTS

Individuals

1. Fred C. Jaronitzky, Jr., Univ. of Illinois
2. Don W. Gibson, California State Polytechnic Institute
3. Charles M. Mead, Iowa State College
4. Thomas E. Quint, Kansas State College
5. Richard A. McKellar, Clemson Agricultural College
6. Lyle Surprise, Univ. of Wisconsin
7. Fred J. Steffens, Univ. of Illinois
8. William O. Lenoir, Mississippi State College
9. Marlin Fouse, Pennsylvania State Univ.
10. Richard F. Flowers, Ohio State Univ.

Teams

1. Univ. of Illinois
2. Iowa State College
3. Mississippi State College
4. Oklahoma A. & M. College
5. Ohio State Univ.
6. Univ. of Wisconsin
7. South Dakota State College
8T. Univ. of Connecticut
8T. Kansas State College
10T. California State Polytechnic Institute
10T. Pennsylvania State Univ.

MILK

Individuals

1. Lyle Surprise, Univ. of Wisconsin 9.17
2. Don W. Gibson, California State Poly. Institute 14.84
3. William Breene, Univ. of Wisconsin 15.00
4. James Dolan, California State Poly. Institute 15.08
5. Charles M. Mead, Iowa State College 15.58

Teams

1. Univ. of Wisconsin 49.17
2. California State Poly. Institute 52.50
3. Pennsylvania State Univ. 57.35
4. Iowa State College 58.25
5. Univ. of Illinois 59.69

BUTTER

Individuals

1. Fred Dryg, Univ. of Minnesota 9.75
2. Charles M. Mead, Iowa State College 9.84
3. Richard F. Flowers, Ohio State Univ. 10.00
4. James E. Turner, Univ. of Nebraska 11.34
5. Gene L. Krajnak, Ohio State Univ. 11.67

Teams

1. Ohio State Univ. 36.17
2. Univ. of Nebraska 38.18
3. South Dakota State College 38.89
4. Univ. of Minnesota 40.50
5. Cornell Univ. 43.84

CHEESE

Individuals

1. Thomas E. Quint, Kansas State College 23.84
2. Thomas Ruzicka, Iowa State College 24.35
3. Ronald Upshaw, Oklahoma A. & M. College 24.83
4. Fred C. Jaronitzky, Jr., Univ. of Illinois 24.84
5. Robert C. Hakeman, Iowa State College 25.00

Teams

1. Iowa State College 75.69
2. Univ. of Illinois 83.33
3. Univ. of Vermont 84.01
4. Pennsylvania State Univ. 85.51
5. Oklahoma A. & M. College 86.18

ICE CREAM

Individuals

1. Ronald Sappington, Univ. of Maryland 27.50
2. Wesley E. Eckert, Washington State College 28.50
3. Jerome J. Donovan, Univ. of Massachusetts 31.34
4. William E. Smith, Univ. of Connecticut 33.00
5. Fred C. Jaronitzky, Jr., Univ. of Illinois 33.17

Teams

1. Univ. of Maryland 104.42
2. Univ. of Connecticut 105.51
3. Mississippi State College 107.34
4. Ohio State Univ. 108.67
5. California State Polytechnic Inst. 112.67

Iowa State Host to Protein Chemists

Forty-eight persons attended the Third Annual Symposium on Milk Proteins held in Memorial Union at Iowa State College, Oct. 26-27. Representatives from Wisconsin, Minnesota, Michigan State, Illinois, Missouri, North Dakota, Kansas State, and Iowa State were in attendance. Ten papers were presented for discussion.

One evening was devoted to a meeting of the American Dairy Science Assoc. Committee on Protein Nomenclature. Dr. E. W. BIRD of Iowa State was in charge of the meeting.

Western Division of A.D.S.A. Names Officers

Officers elected for 1957 by the Western Division, American Dairy Science Assoc., are as follows: Chairman, G. C. ANDERSON, Univ. of Idaho; vice-chairman, I. W. SLATER, Univ. of Wyoming; secretary-treasurer, M. H. EHLERS, Washington State College.

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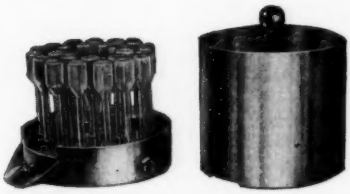

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Nelson Gives Up Headship at Montana

J. A. NELSON, head of the Dairy Industry Department at Montana State College since 1929, has resigned this position effective Nov. 1. For several years Dr. Nelson, the 30th president of the American Dairy Science Assoc., served as dean of the graduate school. He will devote full time to teaching and research.

J. C. BOYD has been named to head the department in Nelson's place. Boyd, a graduate of Washington State, obtained his Ph.D. degree from Michigan State. He is married and has three children.

Meetings Held in Vermont

More than 200 persons attended the 35th annual conference for dairy plant operators and milk distributors, held Oct. 10 and 11 at the Univ. of Vermont. Subjects discussed were plant sanitation and quality and sales of milk. The highlight of the talks was a "Fieldmen's Quality Problems Clinic." Questions were answered by a panel of six scientists.

The annual meeting of the Vermont Dairy Plant Operators' and Managers' Association was held in conjunction with the conference. This organization was started 35 years ago. It has grown from 80 charter members to the present membership of 400.

New Staff Members at Clemson College

CECIL CONLEY joined the Dairy Dept. staff July 16, 1956, as assistant dairy scientist. Dr. Conley received his B.S. and M.S. degrees from the Univ. of Kentucky and his Ph.D. from North Carolina State College with a major in nutrition and a minor in biochemistry. His work at Clemson will be concerned with dairy production research.

R. W. HENNINGSON joined the staff Feb. 23 as assistant dairy scientist. Dr. Henningson received his B.S., M.S., and Ph.D. degrees from Cornell Univ. in the field of dairy industry. His work at Clemson will consist of research on the chemical composition of milk with major emphasis on solids-not-fat.

C. B. REEVES joined the staff Oct. 1 as extension dairy technologist. Mr. Reeves received his M.S. degree from Ohio State Univ. in the dairy technology field and comes to Clemson after practical experience in dairy plant operation in the Philadelphia area. His field work will be to expand the markets of South Carolina dairy products through quality control and the development of consumer acceptance of the fresh milk by-products.

Kentucky Wins International Dairy Cattle Judging Contest

In a field of 16 teams Kentucky placed first in the International Collegiate Dairy Cattle Judging Contest held in conjunction with the International Dairy Show at Chicago October 8, 1956. After Kentucky came Penn. State, Iowa, Kansas, Rutgers, Washington, Ohio, Illinois, Purdue, and South Dakota in the order named. The high individual cattle judge was CHARLES FINK, a student from the Univ. of Illinois.

The high teams and high individual in each of the breeds judged were as follows:

Breed	*Team*	*Individual*	*University*
Ayrshire	Kentucky	Shirley Henrickson	Wash. State
Brown Swiss	Ohio	Max Benne	Michigan State
Guernsey	Kentucky	Charles Fink	Illinois
Holstein	Penn.	Joseph Deighan	Rutgers
Jersey	Wash.	L. C. Garrison, Jr.	Kansas State
Milking Shorthorn	Iowa	L. C. Garrison, Jr.	Kansas State

Iowa Takes First in all Products in Chicago Contest

The dairy products judging team from Iowa State placed first in All Products in the International Dairy Show Products Judging Contest held in Chicago, Oct. 8. This event, held annually in conjunction with the International Dairy Show, was sponsored by the Chicago Dairy Technology Society. Second place went to the South Dakota State team, and Illinois placed third. The high ranking individual in All Products was David Duda of Iowa State. Eleven midwestern teams were entered in the contest.

The high teams and individuals in each of the products judged were as follows:

Product	*Team*	*Individual*	*School*
Milk	Iowa State	Dennis Burchard	Nebraska
Ice cream	Ohio State	Richard Flowers	Ohio State
Butter	Illinois	Fred Dryg	Minnesota
Cheese	Iowa State	Robert Hakeman	Iowa State

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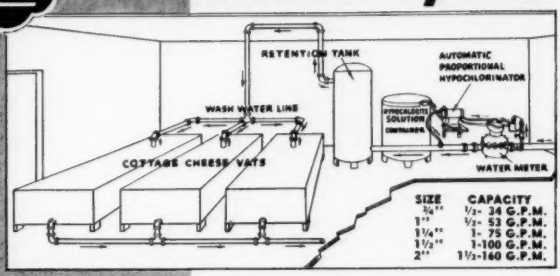

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New Products and Processes Revealed at Dairy Show in Atlantic City

New developments at the Exposition, in Atlantic City's Convention Hall, Oct. 29–Nov. 3, seemed to center on automation — automatic control of mechanical processes. Automation was evident in packaging operations. An almost completely automatic set-up for washing, filling, sealing, and crating milk bottles, and stacking the crates and unstacking them was demonstrated. Only one person was required to handle the entire operation.

Ice cream novelty manufacture also came in for its share of automatic innovations. Several machines handled up to 600 or 700 stick confections per hour. A completely automatic cone filler was exhibited.

Automatic in-place cleaning remained an important continuing trend, with additional refinements in evidence. In-place spray cleaning took the place of conventional hand-brush methods in cleaning of transportation tanks and processor pasteurizers and tanks. Also receiving new attention was farm equipment, with the appearance of refinements on automatic cleaning of pipeline milkers.

Automatic merchandising was not overlooked. Venders of milk and ice cream, in various sizes and types of containers, for both outdoor and indoor distribution, were in evidence. Basic to the automation of all machinery were the automatic controls. Exhibits showed controls for temperature, pressure, timing.

New methods of processing with higher temperature, shorter time pasteurizers, usable for processing of ice cream mix, butter and cream as well as fresh milk were featured. The new high temperatures were said to make it possible to process ice cream mix without a stabilizer. The process can be used for whipping cream, for coffee cream and baby formulas. The makers of the new pasteurizers claim an almost sterile milk at 275° F. Pasteurized by the new equipment at lower temperatures of 218° to 220° F. for 3 seconds, milk has good keeping quality and no cooked flavor, the makers of the pasteurizer claim. Also conspicuous were variations on the vacuum processor for removal of off-flavors in milk. This processing equipment was planned either as auxiliary to or part of a normal pasteurization process.

Adaptability of new machinery and equipment to standard systems and the development of multiple-use equipment were important trends. As an instance, new bottle and capping equipment is adaptable to the filling of all sizes of bottles from half-pint to gallon. New small ice cream cabinets and soda-fountain units can be combined in any number desired to give all the services of a conventional fountain. Small, they can be fitted into a window space for serving customers outside the window. Similar units can form an "island" for service to customers on both sides of the fountain.

Other types of processing equipment making history at the show included:

"Instantizers" to render nonfat dry milk instantly soluble, in either cold or hot water.

Fully automated system for production of ice cream stick novelties, filled cones.

Three-tube continuous freezer for ice cream, which can be so used that each tube contains a different flavor. They can also be operated separately.

Automation remains one of the best answers to what's new in bottle crating and decrating, crate stacking, and destacking equipment. One almost completely automated system was capable of handling the multiple operations of bottle washing, filling, capping and crating, crate stacking and crate unstacking, without attendance by workers.

Equipment to handle new large sizes of containers was in evidence. Bottle and carton fillers and bottle washers for half-gallon and gallon-sizes were offered widely. An ingenious machine placed handles in a pair of quart cartons to create a half-gallon container. In the container field, advances in old materials and use of new materials were seen. Changes in treatment of containers to obtain maximum visibility of product were prominent. Large size containers for milk and other dairy products were common. New developments included:

A new process for strengthening returnable glass bottles, with micro-thin coating protecting the bottle from most abrasions and scratches.

A new single-service milk container with plastic liner, adapted to consumer retail use in half-gallon and gallon size, and made in 3-gallon size for the home milk dispenser, 6-gallon for the commercial dispenser. Made with polyethylene liner and cardboard carrying carton, it is claimed nonbreakable, leakproof, light weight, space saving, and economical. A pour spout makes it easy to use.

Appearance of large numbers of gallon jug and half-gallon jug fillers, cappers and washers, indicating new emphasis on large sizes.

"See-through" features, like all-plastic combinations, plastic lids. Attractive color printing to increase impulse buying.

Appearance of packages attractive enough to be used on the table or even at parties.

Dominating the transportation scene were reduction of empty weight and increased emphasis on insulation and refrigeration qualities. Some of the weight reduction is accomplished by use of new materials. Prominent was the dual purpose delivery truck, which handles combinations of milk, ice cream, cream cheese, and fancy goods. Important to new truck models were innovations for driver convenience, making it possible for the driver to sit

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or stand and to have easy access to his product for handling.

Other developments include:

Aluminum body in panels, lightweight and easy to repair.

Vapor-proof roof for ice cream and frozen foods truck bodies.

Plastic doors and jambs to prevent sweating.

Use of magnesium alloy for weight and modeling advantages.

Squared-up body design to gain increased capacity.

Continuous refrigeration, effected by plug-in or through motors, or by combination of both (effected without opening the trucks).

Fast pull-down and automatic frost prevention in refrigeration systems.

Condenser units that pull out for repair.

Small-package refrigeration units, light weight, taking up little milk bottle space.

In addition to the commercial displays a noncommercial exhibit representing an economical method of disposing of dairy wastes was an example of scientific advance by government and industry working together.

U. S. Civil Service Positions Open

The U. S. Civil Service Commission has announced an examination for filling the positions of Prison Farm Supervisor, $4,080 a year, and Prison Farm Assistant, $3,670 a year, in various federal penal and correctional institutions throughout the United States. The specialized farming operations covered by the examination include field crops, truck gardening, orcharding, dairying, beef production, swine production, poultry production, meat processing, cannery operations, grounds maintenance, and general farming.

No written test is required. To qualify, applicants must have had experience in farming operations including experience in the special field for which application is made. Provision is made for the substitution of pertinent college study for part of the required experience.

Applications will be accepted until further notice and must be filed with the Board of U. S. Civil Service Examiners, U. S. Penitentiary, Leavenworth, Kansas. Further information and application forms may be obtained from the U. S. Civil Service Commission, Washington 25, D. C., or from many post offices throughout the country.

New Germicide Labels Apply in Hard-Water Areas

Manufacturers of germicidal chemicals for dairies, restaurants, and food plants can check on requirements for registering their products for use in hard-water areas in the Sept. 19 issue of the *Federal Register*, the U. S. Department of Agriculture advises. The Register gives a detailed explanation concerning acceptable labeling of such products under the federal Insecticide, Fungicide, and Rodenticide Act.

Large quantities of germicidal chemicals are used in rinses for utensils, dishes, and glassware in compliance with laws protecting the public health. But naturally occurring hard waters can slow down the rate of germicidal activity of some disinfectants and sanitizers, so that they may not perform satisfactorily in all hard-water areas when quick germicidal action is essential. Because of this, the U. S. Public Health Service, Department of Health, Education, and Welfare, last March developed recommended standards of effectiveness for germicides intended for use in hard water. Such products that move interstate must be registered and their labels, list of ingredients, directions, and cautions for use must be accepted by USDA.

Germicide labeling which does not include specific directions for use in hard-water areas will require no change, at least for the present. New labeling of this type will continue to be accepted for registration. However, manufacturers or distributors who submit labeling with specific claims and directions for use of germicides in hard-water areas must submit adequate experimental data to justify proposed claims and representations.

Confronted with the question, "What is hard water?" in devising tests for germicidal products, bacteriologists and chemists in USDA's Agricultural Research Service came up with a standard solution fairly simple to prepare. It has been adapted from a standard established for testing purposes by a large commercial dairy. This formula contains magnesium chloride and calcium chloride as hardening agents. While numerous chemicals cause hardness of water in various parts of the United States, the two chosen for test purposes are regarded as the "problem" chemicals most widely encountered.

Products are tested for germicidal effectiveness in the "standard" hard water, which can be formulated to represent various degrees of water hardness. The number of bacteria must be reduced by 99.999% within 30 seconds in a prescribed test with a lukewarm hard-water solution of the germicide.

Local public health officers, responsible for enforcement of state, county, and municipal requirements, are guided by standards recommended by the Public Health Service. Knowing the hardness of local water, they can judge from label information on germicidal products which formulation will best protect public health in their areas.

Products slowed down in germ-killing power by hard water may continue to be used in soft-water areas, or for jobs where they are in longer contact with surface to be sanitized. Built-in or accompanying water softeners might also increase their effectiveness in hard water.

Every Member Suggest a Member

The membership committee of A.D.S.A. is planning a new campaign for the Association's 51st year. The committee is striving for a membership goal of 2,200 members by the time of the annual meeting in June. The committee urges that each member of the Association suggest one new candidate for membership. Suggestions may be sent to the committee member in charge of an area. Their names and addresses are as follows:

Maine, Connecticut, Vermont, Massachusetts, New Hampshire and Rhode Island — D. J. HANKINSON, Dairy Dept., Univ. of Massachusetts, Amherst.

New York, Pennsylvania, New Jersey, Delaware, and Maryland — J. G. LEEDER, Dairy Dept., Rutgers Univ., New Brunswick, N. J.

Virginia, West Virginia, North Carolina, South Carolina, Georgia, and Florida — P. M. REAVES, Dairy Dept., Virginia Polytechnic Institute, Blacksburg.

Minnesota and Wisconsin — J. J. JEZESKI, Dairy Dept., Univ. of Minnesota, St. Paul.

Michigan, Indiana, Illinois, and Ohio — T. D. HARMAN, Dept. of Dairy Technology, Ohio State Univ., Columbus.

Kansas, Missouri, Iowa, Nebraska, North Dakota, and South Dakota — T. J. CLAYDON, Dairy Dept., Kansas State Agricultural College, Manhattan.

Oklahoma, Tennessee, Texas, Alabama, Mississippi, Louisiana, Kentucky, and Arkansas — J. B. FRYE, Dairy Dept., Louisiana State Univ., Baton Rouge.

Nevada, Utah, Colorado, Idaho, Montana, Arizona, New Mexico, and Wyoming — A. J. MORRIS, Dairy Dept., Utah State College, Logan.

California, Oregon, and Washington — H. A. BENDIXEN, Dept. of Dairy Science, State College of Washington, Pullman.

Canada — R. R. RIEL, Chemistry Division, Science Service, Ottawa; V. S. LOGAN, Animal Husbandry Division, Central Experimental Farm, Ottawa.

Let us all do our part in promoting the growth of the American Dairy Science Association. *Every member suggest a member.*

HAROLD E. CALBERT
Chairman, Membership Committee

Whitlock Joins N. D. Staff

G. P. WHITLOCK has been appointed director, Health Education Service of the National Dairy Council, effective Nov. 1, 1956, according to MILTON HULT, N.D.C. president. Dr. Whitlock will serve also as coordinator of the activities and services of the following N.D.C. departments: Affiliated Unit Service; Library; Materials Development; Program Service; and Nutrition Research. As director of Health Education Service, Dr. Whitlock will work closely with the program personnel of N.D.C.'s 73 affiliated Dairy Council units to aid them in the community nutrition education programs they conduct in 36 states across the nation.

Widely known in the food technology field as a public speaker and author of numerous professional papers, Dr. Whitlock has been associated since 1947 with Merck and Company as technical nutritionist and sales counselor. In this position, his primary responsibility has been to assist the company's promotion departments and salesmen to translate and use technical scientific information as effective sales tools.

Dr. Whitlock was born on a dairy farm in southern Illinois and graduated from Southern Illinois Univ. with a degree of Bachelor of Education. He earned his master's and doctorate degrees at Pennsylvania State Univ., where his studies were concerned with the dairy industry, education, and nutrition science. He has taught at both the high school and college level.

Completed Theses

M.S. Degree:

ANTHONY P. DE LUCA — Relative effectiveness of various antioxidants fed to lactating dairy cows on incidence of oxidized milk flavor and on apparent carotene and tocopherol utilization. Univ. of Connecticut.

C. I. A. LANG — A comparison of methods for determining holding time in high-temperature short-time pasteurizers. Cornell Univ.

Ph.D. Degree:

H. PATRICIA MACLEOD — Leucine metabolism of *Streptococcus lactis* var. *maltigenes*. Univ. of Connecticut.

OUR ASSOCIATION

Off to Another Half-Century

DEAR FELLOW MEMBERS:

The Golden Jubilee is over. The outstanding accomplishments of our first 50 years have been recorded in the June issue of our Journal. We are proud of the great strides made by our industry due to the pioneer spirit that has made America the land of opportunity. We are also proud of the accomplishments of our Association.

At the kick-off of the second half of our century, it appears desirable to take a quick look ahead and see where are are going. In other words, let us take a peak at our objectives. So out of the moth balls comes our constitution to give us our official goals. These are the promotion of scientific research, the improvement of educational methods, the encouragement of worthy intra-industry and inter-industry cooperative endeavors, and the publication of the *Journal of Dairy Science.*

C. F. Huffman

Research. We are in an era of great and rapid changes in the field of dairy science as well as every other field of endeavor. Research is necessary to keep our industry on the march. Research is the life blood of modern industry. Certainly our Association is obligated to stimulate scientific investigations. This can be done in several ways; (1) by attendance at our scientific meetings, where dairy scientists present papers and visit with fellow scientists, and (2) by publishing the results of scientific work in our Journal. These two factors act as a great stimulus to research advancement in the dairy field.

Then, too, the incentive awards administered by our Association tend to encourage scientists to do a better job. The two Borden Awards, one in Manufacturing and one in Production, and the American Feed Manufacturers Award presented each year to outstanding scientists certainly should stimulate our scientists. Other incentives should be developed to enable our industry to keep pace with the times.

Education. Dairy education was the main objective of the small group of pioneers who organized our Association. But objectives, like New Year's resolutions, are of little value unless something is done about them. Over the years we became so involved in research that the education of our youths was almost forgotten. Everyone recognizes the need for good teachers and curricula that are in step with the times. We have failed to take the leadership in this field in the past. The drop in enrollment in dairying in our colleges and universities during the past decade should be of vital concern to all of us. During the past few years steps have been taken to do something about dairy education. We have a committee on education that is vitally concerned with improving the methods of educating our youth. It is just as important to encourage good teaching as it is to encourage good research. As a matter of fact the two go hand in hand. Superior research men need superior instruction and inspiration to excel. We realize the importance of good instruction, but have been slow to do much about it. Our colleges and universities are in need of more good teachers in order to supply the number of well qualified students that industry can use.

The Dairy Science Teaching Award was made available to encourage good instruction. This award is given in alternate years to Production and Manufacturing teachers. This is a step in the right direction — more recognition to outstanding teachers.

Extension. Our dairy extension members are doing a real job of educating the dairymen in the field. Special techniques have been developed for putting across practical knowledge to the dairymen and dairy manufacturers. Many of these techniques have been exhibited at the annual meeting for the benefit of our extension workers.

More rapid progress of the dairy industry depends, to a considerable extent, on the speed with which sound scientific findings are used after their discovery. The greatest impact on our industry today would be greater efficiency of milk production and milk processing at the "grass roots" level.

The DeLaval Extension Award is offered in this field in the appreciation of service rendered by the extension dairymen and dairy manufacturers.

Intra-Industry and Inter-Industry Cooperation. Our constitution gives as one of our objectives the encouragement of worthy intra-industry and inter-industry cooperative endeavors. If our industry is to progress in the future, it is imperative that our Association make full use of our members who are representatives of commercial fields. This means greater use of these members as officers and members of committees. A start has been made in that direction.

The dairy industry needs closer cooperation between various dairy organizations. H. B. Henderson is our representative on the Education Subcommittee of the Dairy Industry Committee of the Milk Industry Foundation. The purpose of this committee is to find out why our

dairy enrollment in colleges and universities is low and what to do about it.

Better relations with United States Public Health Service have been implemented by the appointment of the Public Health Liaison Committee of A.D.S.A., with A. C. Dahlberg as chairman.

We have representatives in several scientific societies: American Association for the Advancement of Science by J. W. Thomas; American Institute of Biological Sciences by H. D. Eaton; and the National Research Council by W. E. Krauss. L. A. Moore is a member of the committee of Inter-Society Cooperation. W. E. Petersen represented our Association at the annual meeting of the National Association of Artificial Breeders last September. C. G. Bradt represented our Association last September at the dedication program at the Plum Island Disease Laboratory, Long Island, N. Y. We have representatives on the Dairy Remembrance Fund. We also have a Joint Committee on Grassland Farming.

We should strive for closer cooperation with *all* related organizations.

Divisions. Another objective is to bring about closer relations with our three divisions. I. W. Rupel, a member of the Executive Board, represented our Association at the meeting of the Western Division in Logan, Utah, in July. Dr. Rupel was very well pleased with the meeting. Our secretary, H. F. Judkins, attended the Eastern Division meeting in September. I am planning to attend the Southern Division meeting in February. It is our feeling that strong divisions of A.D.S.A. will contribute to the advancement of our industry.

Our Journal. An important objective of our Association is the publication of the *Journal of Dairy Science*. Our present editor, P. H. Tracy, has added many new features, including People and Events, Letters to the Editor, Technical Notes, and Our Industry Today. Also, manufacturing and production articles have been separated for the convenience of the reader. Boxed-in forewords at the beginning of each article giving the reader a thumb-nail sketch of the material covered by the article have recently been added. These innovations have done much to improve the Journal and to increase reader interest. My hat is off to Paul Tracy.

Membership. It goes without saying that increased membership will make our Association stronger and more capable of rendering greater service to our industry. It takes a big budget to keep our Association on an even keel. The best way to solve our financial problem is to increase membership. Chairman of our Membership Committee, H. E. Calbert of the University of Wisconsin, is on the ball, but he needs the help of each of us to increase the membership over the 2,000-mark of last year.

Student Affiliate. We have every reason to be proud of the work of W. L. Slatter and his Student Affiliate Committee. More than 400 students representing 33 local dairy clubs in as many states are affiliated members of our Association. The Student Affiliate Committee is developing two new programs for individual dairy students. One will give recognition to outstanding undergraduate and the other to graduate students based on their skill in writing and presenting technical papers.

Another objective seldom listed is greater participation by our members in the affairs of our Association. You can strengthen your organization by making suggestions for the improvement of the Association and by attending the annual business meeting. Feel free to express your opinions through our Journal.

Yours for a bigger and better Association,

C. F. HUFFMAN, *President*

STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Cornell Chapter Sponsors Centennial Celebration of the Founding of the First Butter Factory in U. S.

The first butter factory in the United States was built in Campbell Hall, Orange County, N. Y., in 1856. Conflicting dates and places such as Wallkill, N. Y., 1861, and Manchester, Iowa, 1871, have appeared in a number of textbooks.

As far back as 1917 Dr. E. S. Guthrie, of the Cornell University Dairy Department, gathered facts indicating that the first butter factory was started in the village of Campbell Hall in 1856. He later used this date in his book, *The Book of Butter*, published in 1923. Through many years of correspondence with local and state historians and people associated with the early butter industry, Dr. Guthrie was finally convinced that this was the true date and place of the first butter factory. This year, one hundred years later, sufficient evidence was presented to the historian of the New York State Department of Education to establish this date as being authentic. The University of the State of New York, through the Division of Archives and History, has placed a marker at the spot along New York State Highway 207 to commemorate this historical event.

To help celebrate this centennial anniversary, the Cornell Chapter of the A.D.S.A., under the guidance of Bill Ernst as Committee Chairman, built a replica of a portion of this old butter factory and equipped it with a hundred-year-old churn powered by a horse treadmill, a butter worker, an iron kettle, and miscellaneous small butter-handling equipment as an exhibit for the annual Cornell Farm and Home week, March 1956. Then, in September, they took this exhibit to the New York State Fair.

The Club financed the work of an Orange County historian in gathering data to substantiate this historical fact. They have also paid for a bronze plaque which will be placed on a boulder at the site of this first butter factory. As a final step in the celebration, the Club helped to sponsor a program held in Campbell Hall on Oct. 26. William Saurer, president of the Cornell chapter, presided at this celebration.

Replica of First Butter Factory in U. S. Built in Campbell Hall, New York, 1856. William Ernst, Cornell Dairy Science Club member, and Betty Ann Steer, Dairy Science Club Queen for Farm and Home Week, 1956, pose as buttermakers.

Babcock Dairy Science Club Meets

The Babcock Dairy Science Club opened its first meeting of the year with a talk on the manufacture of rennet by C. A. ERNSTROM. The new members were welcomed into the club. The placings of the Wisconsin Dairy Products Judging Team in the regional contest at Chicago were announced. The team placed first in ice cream, second in cheese, seventh in milk, ninth in butter, and fourth in judging all products. BILL BREENE, president of the club, placed second in ice cream and third in cheese.

Rutgers Dairy Science Club News

After a quiet summer, the Dairy Science Club of Rutgers Univ. has begun another busy year. At the first meeting on Sept. 19, a report on the dairy club exhibits at the annual meeting of the American Dairy Science Association held at Storrs, Conn., in June 1956 was given. The Club entered a scrapbook and a surrounding exhibit at this meeting and won fourth place out of fourteen entries.

The Club welcomed two new staff members in the Dairy Department, who will also assume the role of club advisors. DR. CASON and MR. BAUMGART arrived on campus this fall to take the place of B. R. POULTON, who has gone to the Univ. of Maine.

The year officially began for the Dairy Club when it set up an exhibit at the College of Agriculture's Annual Freshmen Reception, held the evening of Sept. 13. The exhibit concerned judging dairy cattle and attracted the attention of many people.

LETTERS TO THE EDITOR

Presence of α-Ketoglutaric Acid in Milk

A recent note to this Journal *(3)* has reported the presence of α-ketoglutaric acid in milk. The authors indicated that they had made a "reasonably careful search of the literature" and could find no reports concerning this acid in milk. In view of the vast amount of scientific literature which is being published in the world today, use of the term "reasonably careful" seemed well advised. It has been brought to our attention by Virtanen *(4)* that he and an associate have found α-ketoglutaric, pyruvic, and glyoxylic acids in cow's milk. These findings are published as a brief note in a Finnish chemical journal *(2)*. A communication concerning the observations also was sent to the 14th International Dairy Congress, held in Rome, September, 1956.

The undersigned also has received additional information on keto acids in milk from the investigations of Harper *(1)*. Although these data have not been published, it seems well to summarize them at this time in order that the status of the research will be brought up to date. In the analysis of some 40 samples of raw milk from individual cows, α-ketoglutaric and pyruvic acids were identified in all samples. Oxalsuccinic, oxalacetic, acetoacetic, and α-ketoisocaproic were identified in some samples of milk. Other studies have secured presumptive evidence of hydroxypyruvic acid *(2)* and α-ketoisocaproic acid *(3)* in milk.

STUART PATTON
Department of Dairy Science
The Pennsylvania State University

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Note to Ghee Manufacturers

"During last two or three years you might have come across a number of times in newspapers or magazines something about ghee. Essentially it is only dried butter oil with its own characteristic appearance, aroma and flavour. The United States Govt., to my knowledge, has entered into many contracts with several Asian countries like India, Pakistan, Iran, etc., regarding the supply of ghee. At present the manufacture of ghee in the States is gaining more and more importance than ever before. As far as I know many private firms have already obtained contracts for its manufacture and supply to the countries mentioned above.

"In view of its importance and improving demand I would like to take this opportunity to express my opinion through your journal which may be helpful for some of those interested in that business. The difficulties with which the manufacturers in this country are confronted may be listed as below:

"(1) Lack of characteristic flavour and odour: The main reason is due to the fact that the butter which is being used in this country for the manufacture of ghee is generally made from unripened cream.

"(2) Lack of typical granular structure: This quality of ghee more or less depends upon physical and chemical factors like time, temperature of cooling, composition of fat, etc. A greater part of ghee in India is made from buffalo milk, which not only is rich in fat but also of slightly different composition. This certainly affects the size, melting point and amount of granulation in ghee.

"(3) Decolorization of the product: Butter and hence ghee from Indian buffalo is white. However, Indian cow's milk is as yellowish as in U.S.A. Since a higher percentage of ghee is made of buffalo milk, the colour of ghee if white is always preferable.

"With these points in view, it will be a good idea for the ghee manufacturers in the States to produce a product which should be highly desirable in the countries to be sent. I have not tried in this letter to give all the details or recipe for the manufacture of a good sample of ghee but have just touched the problems in general which are bound to be faced by any manufacturer who is not familiar with it."

S. M. HUSAINI
Dept. of Dairy and Food Industries
Univ. of Wisconsin

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ABSTRACTS OF PAPERS PRESENTED AT WESTERN DIVISION MEETING

The annual meeting of the Western Division of A.D.S.A. took place July 15-18 at Reno, Nev. Following are abstracts of the papers presented.

Studies of stabilizers in chocolate drink as related to pilot plant operations. H. S. WILLARD AND W. R. THOMAS, Univ. of Wyoming, Laramie.

Using 20 different stabilizers under a standard laboratory procedure, the authors found wide differences in the minimum amount of stabilizer necessary to hold Dutch process cocoa in suspension. Formulas found satisfactory in the pilot plant produced a nonsettling product under commercial conditions. The authors believed that the pilot plant method described could be used to advantage in any dairy plant with little expense involved. The authors believed also that the pilot plant method was a means of checking costs and in making viscosity changes to meet a particular market demand.

Evaluating the nutrients of milk and milk products. G. A. RICHARDSON, Oregon State College, Corvallis.

A unit system of evaluating the foods within a group of the Basic 7 or 4 recommended in Daily Food Plans is described. A unit of a nutrient is considered as the recommended daily requirement of the nutrient. For example, 3,000 calories of energy, 70 g. of protein, or 1.8 mg. of riboflavin, etc., each would represent one nutrient unit. The sum of the nutrient units represents the nutrient value of a food. The nutrient values of dry whole milk and dry non-fat milk solids are shown to be 18.4 and 22.7 units per pound, respectively. A table shows the calculated unit values of 12 dairy products, the cost per unit and the cost per serving on a unit basis. A comparison of these values with those of Group V of the Basic 7, or Group II of the Basic 4 (Hayes) is shown.

Costs of shipping fluid semen compared with estimated costs of field handling frozen semen. M. H. EHLERS, J. L. ALBRIGHT. and R. E. ERB, State College of Washington, Pullman.

A study was made of the solid CO_2 requirements of a storage chest having approximately 3½ cu. ft. capacity and considered as suitable for field handling frozen semen. An integral electric compressor maintained a –40° C. temperature within the chest. A supply of block CO_2 above the ampules and of chipped CO_2 in the ethanol surrounding the ampules further lowered the temperature to –77° C. Approximately 6 lb. of CO_2 was required per day with such an operation. Without electrical assistance 10½ lb. of CO_2 was used. It was estimated that a chest refrigerated solely with CO_2 was similar in economy to one operating with a compressor and with CO_2 if CO_2 is obtainable at 5 cents per pound or less. The greater initial investment in the electrically assisted chest and the resultant depreciation were considered in this appraisal.

The cost of shipping fluid semen on an alternate day basis was compared with the estimated cost of shipping frozen semen once a month and maintaining a sub-zero storage chest at the inseminator's location. When CO_2 was available at 3 cents a pound the cost of field handling frozen semen appeared to be about the same as the cost of shipping fluid semen. In the area considered (Washington and parts of Idaho and Montana) the securing of CO_2 presented problems in certain localities owing to the distance from the sources of CO_2. Freight and handling charges would be high under such conditions, and supply might be irregular.

Comparison of heated homogenized whole milk, heated skimmilk, and yolk-citrate extenders individually and in combination, on storage motility of bull semen at 40° and –110° F. J. L. ALBRIGHT, M. H. EHLERS, and R. E. ERB, State College of Washington, Pullman.

The effects of combining basic extenders, egg yolk–citrate, heated homogenized milk and heated skimmilk, with and without glycerol, on percentage storage motility at 40° F. was studied. Initially, and on day 3, the nonglycerolated group showed higher values than the glycerolated samples. By day 7 both groups were the same. At the end of 2 weeks storage there was little difference between groups or samples. Egg yolk–citrate and heated skimmilk extenders did not hold up well under storage conditions. However, when these treatments were combined with one another or with heated homogenized milk, compensatory effects were obtained, resulting in an elevation of percentage storage motility in all cases. After 1 week whole milk without glycerol was the best treatment with 40% storage motility. Whenever glycerol was added to a treatment with skimmilk, an elevation in storage motility was observed. There was considerable elevation in glycerol + skimmilk vs. nonglycerolated + skimmilk — 30 to 20%, respectively. Glycerol added to egg yolk–citrate, ½ egg yolk–citrate + ½ heated homogenized milk, and 100% heated homogenized milk resulted in motility declines during storage.

Frozen semen data indicate that satisfactory recovery rates can be obtained by short or no equilibration period. There were slight differences in percentage recovery when freezing at 1.60, 2.65, and 5.02° F. decline per minute. There was also considerable variation in spermatozoa freezability from bull to bull. This factor is perhaps more important than equilibration time and rate of freezing.

The effects of a mono- and diglyceride emulsifier on the Reichert-Meissl number and other properties of ice cream and ice milk. W. R. THOMAS and M. PURKO, Univ. of Wyoming and Wyoming Dept. of Agriculture, Laramie.

Use of emulsifiers in frozen desserts is being recognized by most regulatory officials and is rapidly becoming a common practice among ice cream manufacturers. These emulsifiers are mainly hydrolyzed vegetable fats of mono-, di-, and triglycerides obtained from peanut and cottonseed oils. Not only are the products superior to those used in the past, they are very economical, indicating extensive use in the future.

The effect of these products upon the Reichert-Meissl number and other characteristics of ice cream and ice milk has been studied. Various levels of emulsifiers were added to batches of ice cream and ice milk. The frozen products were then analyzed for fat, moisture, total solids, and Reichert-Meissl number, then examined for flavor and melting ability.

1. Addition of mono,- di- and triglyceride emulsifiers does appear to add to the fat content of ice cream and ice milk when per cent fat is computed on a moisture-free basis. However, when fat content is computed on a wet basis, this trend is not readily detected.
2. Addition of emulsifiers has a tendency to lower the Reichert-Meissl number. However, in these studies this value was not depressed below the limits for normal butterfat.
3. Emulsifier added much in excess of manufacturer's recommendation will tend to produce a frozen product of inferior flavor and melting quality.
4. Ice cream and ice milk to which emulsifiers have been added should be analyzed for fat content by an ether-extraction method.

A study of solids-not-fat variations in different portions of milk drawn. R. H. GARDNER, W. R. THOMAS, and H. S. WILLARD, Univ. of Wyoming, Laramie.

Various portions of milk as drawn from Holstein cows were analyzed for the per cent of solids-not-fat. The general trend was for the middle portion to be higher in solids-not-fat than either the first or last drawn milk on a fat-free basis. The solids-not-fat content of the three portions of milk was shown to be significantly different. Portions of milk from individual cows were found to vary widely in the percentage of solids-not-fat. Morning and evening milk was studied to determine if significant difference occurred between the two in solids-not-fat content. No statistically significant variations were found to exist between the morning and evening milk. The percentage of fat increased as the milking process progressed. There appeared to be more variation between portions with cows in the earlier stages of lactation, than with cows in the later stages of lactation.

A method for determining the total fluid volume of the rumen. T. N. WEGNER, G. M. WARD, and C. V. COLE, Colorado A. and M. College, Fort Collins.

A method is described for determining the total fluid volume of the rumen by means of LiCl dilution. Two liters of LiCl, containing 1,000 p.p.m. of Li, was added to the rumen and samples were taken at 5 or 10-minute intervals up to 1 hour after addition. Samples were filtered, centrifuged, diluted, and analyzed directly on a flame photometer. Between 30 and 60 minutes after addition of LiCl, the dilution method gives results which agree closely with the results obtained by direct measurement of the fluid. Results prior to 30 minutes were erratic, probably indicating incomplete mixing of the rumen contents up to that time.

LiCl did not appear in the blood in significant amounts until 45 minutes after the addition of the solution to the rumen.

Some observations of the effects of management practices upon the flavor of milk. G. M. WARD and B. J. DEMOTT, Colorado A. and M. College, Fort Collins.

This study was made on milk from the college herd, and all milk was tasted by a panel of at least three judges. Usually, the milk was collected from individual cows. The results of this study can be summarized as follows:

1. Age, stage of lactation, and level of production had no effect upon milk flavor.
2. The milk from cows treated for mastitis did not seriously affect the flavor of the herd milk supply.
3. The time of feeding alfalfa silage with respect to milking time had no influence on milk flavor.
4. Heavy molasses feeding was not detrimental to milk flavor.
5. Milo fed as 75% of the grain ration had no detrimental effect upon the milk flavor.
6. It seems unlikely that chlorine or quaternary ammonium solutions are a factor in off-flavored milk in the amounts and strength that would accidentally appear in the farm milk supply.

The microflora of bovine semen. M. S. TRUEBLOOD, S. M. MORRISON, and H. J. HILL. Colorado A. and M. College, Fort Collins.

Semen samples collected with an artificial vagina at the Colorado A. and M. Artificial Breeding Center were examined for motility, morphology, and microbial types and numbers.

To determine the effect of storage upon semen treated with penicillin (750 units/ml.) plus streptomycin (500 γ/ml.), plate counts were run before and after storage at 5° C. for 48 hours.

The organism found most often was the genus *Bacillus* and in order of decreasing frequency were coliforms, *Micrococcus, Proteus, Streptococcus, Pseudomonas,* actinomyces, yeasts, *Serratia* and *Salmonella.* No bull showed a consistent pattern. Since all microbial types isolated, with the exception of *Salmonella,* have their usual habitat in the soil or animal surfaces, it would seem that most, if not all, the organisms isolated were external contaminants added to the semen samples during collection.

Even though no special sanitary precautions were taken at collection the total plate counts (30°) of undiluted semen varied from less than 1,000 to 1.1×10^6 organisms/ml. The average of the counts obtained was 92,000 bacteria/ml. The average plate counts of diluted semen samples immediately after collection and addition of antibiotics was 6,600 bacteria/ml. After storage at 5° C. for 48 hours the bacterial numbers in these samples varied from less than 1,000 to 150,000 bacteria/ml. Some semen samples showed a decline in bacterial numbers while others showed a decided increase.

Metabolic behavior of bovine spermatozoa as influenced by bacteria. M. S. TRUEBLOOD, M. L. HOPWOOD, S. M. MORRISON, and H. J. HILL, Colorado A. and M. College, Fort Collins.

Since the procedure of semen collection for artificial insemination leads to microbial contamination, it may be of great importance in studies on semen to understand the interrelationships between microbial and semen metabolism.

By using the Warburg microrespirometer to measure oxygen uptake it was shown that bovine seminal plasma has little or no metabolic activity. *Bacillus* or *Pseudomonas* organisms added to semen samples produced an oxygen uptake in 3 hours that was significantly higher than that of semen samples alone or that combined oxygen uptake of semen plus organisms. This indicated a metabolic stimulation to either the sperm cells or bacteria or both. By adding fresh sperm cells to filtrates of incubated bacteria plus semen, it was shown that this stimulation was due to the ability of the sperm cells to utilize bacterial intermediate metabolites. Streptomycin was shown to have little or no effect on the metabolism of sperm cells or on *Pseudomonas* organisms, but it was significantly inhibitory to the respiration of a *Bacillus* species.

Fluorosis studies with dairy cows: A progress report. The first forty months on experiment and for the first lactation. G. E. STODDARD, J. L. SHUPE, D. A. GREENWOOD, M. L. MINER, G. Q. BATEMAN, L. E. HARRIS, H. M. NIELSEN, and T. V. BISHOP, Utah Agricultural College, Logan.

Holstein heifers were placed on diets containing various levels of fluorine (sodium fluoride) at 4 months of age. Treatments included two animals on each of the 16 possible combinations of four fluorine levels, two rates of grain feeding, and two levels of a calcium-phosphorus supplement. Fluorine levels included basal (10-12 p.p.m.), 25, 50, and 100 p.p.m. based on hay intake. Grain levels included 1 and 1⅓ recommended amounts during lactation with 2 and 4 lb., respectively, during nonlactation periods. Ca-P supplement was fed at 1% and 3% of grain ration.

During the first 40 months no treatment effects were observed in height at withers, body weight, or reproductive performance. Rib fluorine levels and tooth classification were influenced by fluorine treatment. Heifers on high grain intake produced more milk than those on standard intake. Ca-P intake had little or no influence on heifers. Animals on 100 p.p.m. F showed lameness and exostosis not observed in heifers on other F levels and were "off feed" more frequently. Cows consumed 0.26, 0.54, 1.01, and 1.92 mg. F/kg. body weight for the basal, 25, 50, and 100 p.p.m. groups, respectively. Trial will continue through at least four lactations.

A study of some factors related to rancidity in pipeline milkers. B. J. DEMOTT and L. R. BRANDT, Colorado A. and M. College, Ft. Collins.

A vacuum was drawn on a 4-foot column of 45 mm. glass and raw milk was drawn into this and treated by bubbling various amounts of air into the milk at various levels of vacuum. Surface tension measurements were made, and organoleptic scores were given on each sample of milk after treatment and storage for different lengths of time. The data show:

1. A correlation coefficient of 0.44 between the surface tension value of 90 samples of milk and the degree of rancidity as measured by taste.
2. That the fat content of milk has very little if any effect upon the surface tension value of milk.
3. That the longer the length of storage of milk, with or without treatment, the lower the surface tension becomes.
4. That the longer the time of exposure to the treatment in the "milker," the lower the surface tension becomes.

5. That different milks react differently toward the treatments. This could not be explained on the basis of fat content of the milk.
6. That the greater the vacuum level used in the "milker," the lower the surface tension of the milk will become.
7. That the greater the volume of air passing through the milk, the greater the fall in surface tension of the milk.

Diethylstilbestrol as a treatment for anestrum in dairy cattle. K. R. JOHNSON, Univ. of Idaho, Moscow.

Twenty-three anestrum cows, in which a rectal examination indicated the condition as being due to a retained corpus luteum, were given either 50 or 75 mg. of diethylstilbestrol intramuscularly. The amount administered was determined by the size and tone of the corpus luteum. When the corpus luteum was ½ cm. or larger in width and firm to the touch or if it was enclosed or imbedded in the ovary, 75 mg. of stilbestrol was administered. When the corpus luteum was less than ½ cm. in width and not greatly enclosed in the ovary, 50 mg. was administered.

All of the cows were in estrus within 24 hours after treatment and all had subsequent normal regular estrous cycles. Nineteen of the 23 cows are pregnant or have calved and the remaining four have passed over three or more estrual cycles since last breeding. Thirty-three services were required for a breeding efficiency of 69%. There were no cases of prolonged estrus, abnormally frequent estrus, or other complications from the treatment.

The importance of heredity and environment in causing variation in D.H.I.A. records made in Idaho. K. R. JOHNSON, D. O. EVERSON, and W. R. TAYLOR, Univ. of Idaho, Moscow.

A total of 2,025 daughter-dam comparisons from 237 sires for the three breeds, Guernsey, Holstein and Jersey, were analyzed to obtain estimates of heritability for milk and butterfat production. These estimates were obtained by doubling the intrasire regressions of daughters on dams.

The results of this study indicate that the heritability of milk and butterfat production on a single record basis, for these D.H.I.A. data was 0.264 for milk and 0.302 for butterfat. These estimates are the pooled weighted averages for the three breeds. The estimates of heritability for milk and butterfat production were 0.362 and 0.320 for the Guernsey, 0.260 and 0.220 for the Holstein, and 0.212 and 0.388 for the Jersey breeds.

The maximum likelihood method was used to estimate the genetic and environmental changes in milk and butterfat production for the Jersey and Holstein breeds on a within breed and regional basis.

The Holsteins of the combined regions had genetic regressions of -76.0^{*} lb. of milk on year and -0.25^{*} lb. of butterfat on year. The environmental regressions were $+192.0^{**}$ lb. of milk on year and $+5.97^{**}$ lb. of butterfat on year. The Jerseys of the combined regions had genetic regressions of -27.0 lb. of milk on year and 0.01 lb. of butterfat on year. The environmental regressions were $+72.0^{**}$ lb. of milk on year and $+2.42^{**}$ lb. of butterfat on year.

Environmental changes accounted for large, significant, positive regressions for milk and butterfat production in all regions within breeds.

Trace minerals in the blood and excreta of dairy cows at parturition: A preliminary report. T. H. BLOSSER, W. H. ABBITT, M. F. ADAMS, and G. M. WARD, State College of Washington, Pullman.

Data are available on the trace mineral content of the blood and excreta from ten cows from which blood samples were taken and 24-hour specimens of excreta collected at intervals from approximately 30 days prepartum to 30 days postpartum. Four cows were mature Jerseys, two were primiparous Jerseys, two were mature Holsteins, and two were mature Guernseys. Trace minerals were determined by spectrographic methods. Copper, lead, manganese, and zinc levels are reported in this paper. Whole blood copper values tended to be low at the time of parturition and somewhat lower after parturition than before. Urinary copper increased as parturition approached and decreased during the first 10 days after calving. Fecal copper was lowest from 4 to 15 days after calving. Behavior of lead in the blood, urine, and feces was more erratic than that of the other minerals. Whole blood lead values were at their highest point from 5 to 2 days prepartum and were quite low from 1 day prepartum to 15 days postpartum. Urinary lead was highest, on the average, from 2 days prior to 2 days subsequent to parturition. Fecal lead values were quite variable but highest on the day of parturition. Manganese levels of the blood were low from 25 to 8 days prepartum, increased at the time of parturition, decreased immediately thereafter, and then increased until 25 days postpartum. Urinary manganese levels were highest on the day of parturition and lowest 1 day postpartum. Fecal manganese was very consistent. Whole blood zinc increased gradually to a high point 5 days prepartum and then decreased to a low point 1 day prepartum. After this there was a gradual increase until 5 days postpartum followed by a leveling off. Urinary zinc values were below average from 1 day prepartum

* Significant at the 5% level.
** Significant at the 1% level.

until 5 days postpartum and was high from 25 to 8 days prepartum and from 8 to 25 days postpartum. Fecal zinc was consistently high from 15 days prepartum to parturition and only half as high from 1 to 15 days postpartum.

Comparison of glycolytic activity of first and second ejaculates of bull semen: A progress report. N. S. KUSHWAHA, M. H. EHLERS, and R. E. ERB, State College of Washington, Pullman.

In this study fructose utilization and lactic acid formation are being compared in first and second ejaculates. Data on 12 bulls of the Angus, Guernsey, Holstein, and Jersey breeds are included. The bulls ranged in age from 1 to 7 years at the beginning of the experiment. The present report included 264 first and 264 second ejaculates.

Among the bulls over 18 months of age the second ejaculates averaged *(a)* 27% more in volume than the first ejaculates; *(b)* 34% less in sperm density, *(c)* 5% less in initial motility, *(d)* 2% less in motility after 1 hour incubation, *(e)* 13% more fructose initially, *(f)* 11% less fructose utilization/ml. semen during incubation, *(g)* 28% more fructose utilization/billion sperm, *(h)* 16% less initial lactic acid, *(i)* 8% less lactic acid accumulation/ml. semen during incubation, *(j)* 37% more lactic acid accumulation/billion sperm.

In the bulls under 18 months of age the second ejaculates averaged *(a)* 20% more in volume than first ejaculates, *(b)* 23% less in sperm density, *(c)* 15% less in initial motility, *(d)* 3% less in motility after incubation, *(e)* 2% less fructose initially, *(f)* 24% less fructose utilization/ml. semen during incubation, *(g)* 2% less fructose utilization/billion sperm, *(h)* 4% less initial lactic acid, *(i)* 24% less lactic acid accumulation/ml. semen during incubation and *(j)* 6% more lactic acid accumulation/billion sperm.

ABSTRACTS OF PAPERS PRESENTED AT EASTERN DIVISION MEETING

The following abstracts are from papers presented at the 30th annual meeting of the Eastern Division of A.D.S.A., held Sept. 17 at the Eastern States Farmers' Exchange, West Springfield, Mass.

Flavor research in the food industry. JEAN F. CAUL, Arthur D. Little, Inc., Cambridge, Mass.

Flavor is the problem child of the food industry. Unknown by some, recognized yet forgotten or neglected by others, it has become a major factor to contend with in food production, processing, distribution, and marketing. The problem of survival in this competitive field depends on the consumer, and flavor gives the leading products of the food industry the edge over their competitors. Rules of thumb for appealing flavor are: *(a)* An immediate impression of a high level of pleasant flavor; *(b)* A rapid development of blended flavor having interest factors and no inappropriate notes; *(c)* A quick cut-off of flavor after swallowing with no lingering aftertaste or afterfeel. Flavor analysis is a first step in determining and obtaining flavor appeal of a product. Using the flavor profile method, a trained laboratory taste panel can yield written records of all the flavor properties of any product. These can then be applied to improving existing products or to the creation of new ones. The current literature reflects only a portion of the flavor being carried out or sponsored by the food industry; much is retained as trade secrets. Companies who pay attention to flavor and flavor research are the companies who are getting and keeping ahead in the food industry.

Relative effectiveness of various antioxidants fed to lactating dairy cows on incidence of copper-induced oxidized milk flavor and on apparent carotene and tocopherol utilization.[1] A. P. DELUCA, R. TEICHMAN, J. E. ROUSSEAU, JR., M. E. MORGAN, H. D. EATON, H. P. MACLEOD, M. W. DICKS, and R. E. JOHNSON, Storrs Agr. Exp. Sta., Storrs, Conn.

Recent reports from this station indicated that N,N′ - diphenyl - para - phenylenediamine (DPPD) was effective in preventing the occurence of copper-induced oxidized milk flavor when fed to lactating dairy cows and may possibly have stimulated utilization of carotene. In view of the present availability of many other antioxidants it was decided to test their relative potency in preventing oxidized flavor and in increasing fat-soluble vitamin utilization. Twenty-four cows, 12 Holsteins and 12 Guernseys, were selected for producing milk that would exhibit oxidized flavor 72 hours after the addition of 5.0 p.p.m. of Cu^{++}. After a 1-week standardizing period, the cows were fed no antioxidant; N,N′-diphenyl-para-phenylenediamine (DPPD); 2,6-di-tertiarybutyl-4-methyl phenol (BHT); 1,2-dihydro-2,2,4-trimethyl-6-ethoxyquinoline (Santoquin); *d-α*-tocopherol (Myvamix) at a daily rate of 0.01% of 90% dry matter ration and 2-methyl-1,4-napthoquinone (Menadione) at a daily rate of 25 mg. for a 6-week comparison period. The following results were noted: *(a)* Fat percentage of the milk was slightly increased by the feeding of DPPD and Menadione and slightly decreased by the feeding of Santoquin; *(b)* total tocopherol values of the blood plasma and milk were apparently increased by the supplementation with antioxidants; *(c)* none of the antioxidants fed were as effective as DPPD in inhibiting the incidence of copper-induced oxidized milk flavor. No protection was afforded by any antioxidants except DPPD in the presence of 5.0 p.p.m. of Cu^{++} and only partial protection by Myvamix and Menadione in the presence of 1.0 p.p.m. of Cu^{++}.

[1] This study was supported in part by grants from the Chas. H. Hood Dairy Foundation, the American Cyanamid Co., and the Chas. M. Cox Co. The Monsanto Chemical Co. and Distillation Products Division of the Eastman Kodak Co. kindly supplied some of the supplements.

Solids-not-fat percentage in milk: Its repeatability in consecutive lactation records and its within breed variation among cows. C. J. WILCOX, K. O. PFAU, R. E. MATHER, and J. W. BARTLETT, Rutgers Univ., New Brunswick, N.J.

Monthly milk samples taken during the past 3 years from the Holstein and Guernsey herds of the Dairy Research Farm, Sussex, N. J., have served for the compilation of average solids-not-fat (SNF) percentages on lactation bases. Until June 1955, SNF values were calculated from specific gravity, as determined by the Quevenne lactometer, and from fat percentage, as determined by the Babcock test, by the use of AOAC tables. From June 1955 on, the Watson Pattern lactometer was used exclusively and SNF percentage calculated from tables developed by the USDA. A correlation of 0.919 (highly significant) existed between results obtained by the two lactometers with no difference in their respective SNF means. Seventy cows, 51 Holsteins and 19 Guernseys, had two complete lactation records and were included in the study. An analysis of variance showed a highly significant ($P = 0.01$) difference between breeds, a significant ($P = 0.05$) difference between consecutive lactations, and a highly significant ($P = 0.01$) difference among cows within

breeds. The variance among cows excluding breed differences amounted to 70.5%. The estimate of repeatability of SNF percentage within cows on a basis of consecutive lactations was 0.804.

Preliminary observations on the fetal uterus, ovaries, and adrenal glands in cattle. J. S. GREENSTEIN and R. C. FOLEY, Univ. of Massachusetts, Amherst.

These observations were based on fetuses from cattle slaughtered at known stages of pregnancy. Fetal ovaries, uteri, and adrenals were obtained from five female fetuses aged 124, 130, 139, 148, and 202 days. Two additional fetal adrenals were taken from male fetuses aged 137 and 165 days. In the ovary at 130 days, the region of the rete ovarii and the deposition of the secondary cortex were well defined. At 148 days, the continued growth of the septula forming the tunica albuginea was evident along with the presence of ova. By 202 days, the gland displayed numerous primary follicles and Graafian follicles. The fetal uterus at 124, 130, and 148 days consisted of a relatively undifferentiated mucosa, tunica muscularis, and serosa. By 202 days, uterine glands had not appeared but vascularization of the endometrium and differentiation of the myometrium were apparent. In the fetal adrenal, the glomerulosa was the first cortical layer to differentiate as seen in the 124-day tissues. The fasciculata emerged soon after and was easily recognized in the 148-day fetus. Clusters of medullary chromaffin cells were also apparent within the central portion of the gland at this time. Both cortex and medulla underwent continued growth and differentiation and were very clearly delimited by 202 days.

Centralized processing of dairy records for northeastern United States. S. J. BROWNELL, Cornell Univ., Ithaca, N. Y.

Electronic business machines now make possible centralized processing of Dairy Herd Improvement records. The Extension Division of Animal Husbandry Dept., Cornell Univ., has established facilities and worked out procedures for such a service. The New York Dairy Herd Improvement Cooperative, Inc., has developed its organization with 100% affiliation of all local D.H.I. cooperatives in the state. The state cooperative has a Memorandum of Understanding with Cornell Univ. to process their records. This cooperative offers central processing so that acceptance by an individual member, a supervisor's circuit, or an entire county cooperative will be entirely voluntary. Individual and local affiliating groups are responsible to the state association for all financing and compliance with the national D.H.I. rules. The University will bill the New York D.H.I. cooperative monthly at the rate of 8½¢ for each cow record processed. The New York State Association will bill each county cooperative 10¢ a record, which will include cost of forms, mailing, clerical processing, financing, and State Association operating costs. The same service will be available to any responsible state organization in northeastern United States as soon as it is demonstrated that the program is operating satisfactorily. This may be possible by January 1, 1957. Agreements of state organizations to assume financial and regulatory responsibilities will be made with the New York D.H.I. Cooperative, Inc., who will in turn be responsible to Cornell Univ.

Dairy cow type evaluation. R. A. CORBETT, Univ. of Maine, Orono.

An attempt is being made to develop a program and a "usable tool" to assist dairy farmers in the improvement of type in their herds. Committees for each breed in an area served by a unit of the artificial breeding association are set up consisting of the breeding association bull committeeman for each breed, another dairyman keeping the same breed, and the county agent. (The county agent will eventually be replaced by a third breeder.) These committees are trained by the extension dairyman and a fieldman of the breeding cooperative. Only A.B. daughters which have freshened in D.H.I.A. herds have been type evaluated. A numerical system is used in the evaluation of each part of the animal as well as the over-all type rating. Results to date indicate that these farmer committees can do an effective job of type evaluation. Approximately 25 A.B. daughters of a sire appear to be necessary to indicate a trend in the type transmitted by the sire. Production records of each animal evaluated are recorded on the type sheet.

The effects of pasteurization temperatures and exposure to artificial light on homogenized milk held in cold storage. A. C. SMITH and H. PATRICIA MACLEOD, Univ. of Connecticut, Storrs.

Samples of winter and summer homogenized milk, pasteurized from 163° F. to 188° F. in 5° F. increments by the H.T.S.T. method, were bottled in clear glass, stored at 40° F. in the dark and under 20 ft-c. of incandescent light for 0-, 1-, 2-, and 3-day periods. Ascorbic acid oxidation and degree of oxidized flavor development were less and progressed at a less rapid rate in the samples stored in the dark than in corresponding samples exposed to light. Increasing the temperature of pasteurization increased ascorbic acid retention and decreased susceptibility to oxidized flavor development. Raising the temperature of pasteurization to a point where cooked flavor is not objectionable will tend to counteract these effects of light.

Rebuilt and newly equipped plant laboratory. B. A. LARSON, Supplee-Wills-Jones Milk Co., Philadelphia.

A fairly large dairy plant laboratory, averaging 600 plates per day, and proportionate

other work, was redesigned, rebuilt, and newly equipped for greater efficiency. Efficiency was measured in terms of output per man-hour, accuracy, and breakage and other waste. Layout was improved to reduce steps; air conditioning was installed for comfort and better hardening of plates; the electric supply was vastly increased; much modern equipment was installed, of which the most important are: large agar tempering bath; 35 cu. ft. refrigerator; electric ovens on "Skipper" timers; "Cyclamatic" autoclaves; all-stainless-steel 5-g.p.h. still for easy cleaning; a Heinicke washer that rapidly removes agar and wax pencil markings; and a new convenient, simple, and economical method of handling patrons' samples. Results were a near-halving of labor costs, reduction of breakage by over 80%, improved accuracy, and an increased gross output.

Why so few dairy technology graduates? H. F. JUDKINS, secretary-treasurer A.D.S.A.

Reasons advanced for so few dairy technology graduates in recent years are as follows:

(a) Poor job of recruitment of secondary school graduates.
(b) Demand for engineers, physicists and chemists.
(c) Poor handling by industry — lack of adequate training programs.
(d) Hard physical work.
(e) Lower salaries than many other fields.
(f) Possible inadequate curriculum.

Courses taken by 19 dairy technology students (1955 or 1956) in nine northeastern state universities showed plenty of flexibility and for the most part a minimum of straight agricultural courses required.

It is concluded that the curriculum is not obsolete and an unimportant factor in the scarcity of dairy technology graduates.

THE POSSIBILITY OF COPPER-INDUCED OXIDATION OF MILK IN STAINLESS STEEL–WHITE METAL SYSTEMS[1]

E. W. LUSAS,[2,3,4] E. W. BIRD, AND W. S. ROSENBERGER
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Samples of the first 30 gallons of milk through a white metal-stainless steel system were examined organoleptically and for Cu, Fe, and Ni contents and thiobarbituric acid values. Two components of the oxidized flavor that developed are described — one which followed the skimmilk fraction and the other which followed the cream phase. *Editor.*

During recent years, oxidized flavors have become prevalent in both creamline and homogenized milks, during both summer and winter, in Iowa. A number of factors have contributed to greater incidence of oxidation in milk in Iowa: Adoption of milk ordinances have resulted in the production of lower-count milks; the industry has changed to every other day or three times per week delivery, causing the milk to be held for longer periods before delivery; and in-place cleaning practices have been adopted. During the period embracing these changes, milk-handling systems have in the main become combinations of stainless steel sanitary tubing (SSST) and white metal (WM) fittings. These systems seem to have been accepted without question.

Copper dissolved from WM fittings in SS-WM systems might be a contributory factor causing oxidation in Iowa milk, particularly since Krukovsky and Guthrie *(28)* have indicated that 0.1 p.p.m. Cu in milk can cause tallowy flavors to develop in 24 hours at 0 to 5° C. if the milk has not been depleted of ascorbic acid. This study is a preliminary investigation to attempt to determine whether or not the above hypothesis has any validity.

EXPERIMENTAL PROCEDURES

Milk samples were obtained from the market milk plant of the Dairy Industry Department on the same day each week during a 10-week period (April 5 through June 7, 1955); this period included samples produced during dry feed and pasture feeding periods.

The milk was received in milk cans and was transported from the intake to the raw milk storage tank through an all SS system about 50 ft. in length

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[2] Abstract of a thesis submitted in partial fulfillment of the requirements for the degree Master of Science in Food Technology (Dairy Industry), August, 1955.

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(including a weigh tank, rotary pump, clarifier, and plate cooler). Between the raw milk storage and the can filler the system was about 200 ft. in length (including two centrifugal pumps, one timing pump, a standardization vat, pasteurizer balance tank, a homogenizer operated without pressure, and a HTST plate pasteurizer). The copper-bearing items in the latter part of the system were: three WM valves, one WM adapter, a WM plug, WM homogenizer valve outlet fitting, two WM nipples, and a tinned-copper, homogenizer blow-out disc.

The raw milk samples were collected in an all SS drip-sampler at the highest point in the system, just ahead of the raw milk storage tanks. Pasteurized milk samples were of two types, namely *(a)* those pasteurized by the HTST procedure (72.8° ± 0.5° C., 16 seconds, about 18 hours after receipt; storage of raw milk was at 4.4° C.) and *(b)* those pasteurized by the low temperature–long time (LTLT) method (61.7° ± 0.5° C., 30 minutes; raw milk from drip sampler) in a laboratory pasteurizer built to simulate spray-vat pasteurization.

The HTST samples were representative composites of the first 30 gal. of milk through the part of the system between the raw milk holding tank and the can filler; the milk was caught in 10-gal. SS cans. No dairy product passed through the system prior to this 30 gal. of milk. This system of sampling the HTST pasteurized milk was adopted for two reasons, first to minimize interference with normal plant operation, because generally other products were processed before either creamline or homogenized milk and, second, to permit a greater possibility of finding measurable increases of copper in the samples.

Laboratory "separation" of milk into "cream" and skimmilk fractions was accomplished by centrifuging 250-ml. lots of milk in a size 2 International centrifuge at 1,800 r.p.m. for 30 minutes. The centrifuged samples were chilled at 4.4° C. to harden the cream layer. When solid, the cream layer was tipped on edge and the nonfat portion was strained through cheesecloth. The solid cream layer was washed with three 10-ml. portions of ice water and drained.

Metal-free water (MFW) was obtained by redistilling a distilled water which had been condensed in a block tin system. Before redistilling, 15 ml. of of an H_2SO_4-$Na_2CR_2O_7$ solution (5% v/v H_2SO_4 and 5% w/v $Na_2Cr_2O_7$) were added per 18 l. distilled water to destroy organic materials. The first and last quarters of the distillate were discarded. The still employed was constructed of Pyrex glass except for the condenser inner tube, which was clear quartz.

A Beckman, model DU, quartz spectrophotometer equipped with a red-sensitive phototube and tungsten lamp was employed in determining the 2-thiobarbituric acid values; the trace-metal determinations were made with a Coleman, model 11, Universal spectrophotometer (PC-4 filter). Square Pyrex cuvettes were used with each instrument. Cuvettes were matched for use at 520 and 535 mμ with an aqueous solution of $KMnO_4$ (0.025 g/l) and at 420 and 465 mμ with a 95% ethanolic $CrCl_3$ solution (3.5 g/l).

Metal standard solutions were prepared from a Hilger copper electrode, electrolytic iron[5] and Mond nickel shot.[5] In each case the metal was washed

[5] Obtained through the courtesy of Dr. H. Diehl, Dept. of Chemistry, Iowa State College.

with ethyl ether, 1:1 HCl in MFW, MFW, and dried. Solution was effected over a steam bath in 30 ml. 1:1 HCl in MFW by addition of successive small amounts of 30% H_2O_2. Concentrations of the stock solutions were verified by standard methods [copper *(31)* and iron *(25)* iodometrically and nickel as the dimethylglyoxime precipitate *(39)*].

Some deviations from the methods for determining copper, iron, and nickel (in milk) were employed in establishing the standard regressions. A small piece of litmus paper was employed to establish the pH to which the copper and iron solutions were taken with NH_4OH. Nickel color complexes were formed directly with the standards (extraction was omitted). Regression equations were calculated by the method of Gore (*15*). Plotted curves were not linear on semilogarithmic paper, possibly because of the optical system in the Coleman model 11 spectrophotometer *(1)*. It was considered satisfactory to fit two equations to the plotted data *(3)*; the sections to which these were fitted were selected by observation of the plotted data.

Milk samples were wet ashed by a modified combination of the methods of Smith *(35, 36)* and of Kagi *(21)*. The ashing apparatus was essentially that of Smith *(36)*, except that the condensing funnel within the neck of the digestion flask was not employed and a standard taper was placed in the vertical section of the arm of the fume head, just above the stopper in the suction flask, for ease of dismantling. The modifications made in the Kagi procedure were: Fifty g. of milk[6] was digested with 100 ml. of acid mixture (40 vol. reagent 70% $HClO_4$ and 60 vol. reagent conc. HNO_3) on a variac-controlled hot plate at 175 ± 5° C. until the mixture was clear and a white vapor formed. The hot plate temperature was raised to about 250° C. and the ashed samples were taken to dryness. It was necessary to take completely to dryness to prevent interference in the formation of color complexes of the several cations investigated.

Aliquots of ash solution equivalent to 30 g. of milk were employed for the copper and iron assays and to 200 g. of milk for the nickel assays.

Iron and copper were analyzed by an adaptation of the method of Ventura and White *(38)*. The modifications made were as follows: the acidic ash solution was made alkaline with ammonium hydroxide to incipient phosphate precipitation[7] and then was acidified with 6 *N* reagent HCl until just clear. Three ml. of strong[8] acetic acid-sodium acetate buffer (0.63 mol. redistilled glacial

[6] Two 100 g. samples each were ashed with 200 ml. acid mixture for the nickel determination.

[7] Ventura and White *(38)* employed *p*-nitrophenol as the indicator. With the larger amounts of iron in milk, a ferric-*p*-nitrophenol complex evidently is formed. This complex was orange-yellow, in contrast to the bright yellow alkaline color of the indicator. The color could not be dissipated by addition of acid nor decolorized by the reducing agent. Phenolpthalein yielded similar results. The calcium phosphate precipitate served satisfactorily as an indicator.

[8] The ratios of the reagents in this buffer are those suggested by Walpole *(6)*. The total concentration is five times that of the Walpole buffer. This increase in buffer capacity was found necessary to maintain a pH of 4.4 under the analytical conditions employed.

acetic acid and 0.37 mol. reagent sodium acetate per liter) were added to maintain a pH of 4.4. Copper diethyldithiocarbamate was extracted with successive 5-, 3-, and 2-ml. quantities of ethyl ether–amyl alcohol mixture. It was necessary to filter dispersed ether–amyl alcohol mixture from the aqueous solution (containing ferrous-bipyridyl), after having been made to volume, through a small plug of nonadsorbent cotton (the first 5 ml. were discarded).

Nickel was determined spectrophotometrically as the dimethylglyoxime by the method of Claassen and Bastings *(5)*, except that the nickel was determined in a solution equivalent to 200 g. of milk, and the final volume of the solution read was 50 ml. instead of 100 ml.

Milk samples (for scoring) were placed in half-pint milk bottles (one for each scoring period) and were stored in a household-type refrigerator at about 4.4° C. They were scored at 1, 2, 3, 4, 6, and 8 days after receipt of the milk by a panel of three experienced judges. The first scoring was approximately 7 hours after the milk had been processed. The samples were tempered to approximately 22° C., coded, shaken to redistribute the fat, and presented to the panel. They were scored in accordance with the scorecard (Figure 1) pre-

Degree of defect

10. No oxidized flavor	5. Strongly oxidized
9. Flat	4. Metallic
8. Cardboard or cappy	3. Strongly metallic
7. Slightly oxidized	2. Slightly tallowy
6. Definitely oxidized	1. Strongly tallowy

FIG. 1. Scorecard employed in recording the degree of oxidation that had developed in the milk.

pared jointly by the members of the panel. The judges were asked to score as regards *(a)* maximum oxidative deterioration and *(b)* degree of oxidized flavor development not greater than "strongly oxidized" (score of 5) in order to attempt to determine whether or not the 2-thiobarbituric acid (TBA) test seemed to correlate better with the over-all oxidative defect than with its "oxidized" flavor component or vice versa.

TBA values of the milks were determined by the method of Dunkley and Jennings *(11)*. They were expressed as optical densities of the solutions read.

RESULTS AND DISCUSSION

The accuracies of the analytical methods, as employed in this laboratory, were evaluated by determining recoveries of the metals added to milk samples. The average recoveries were: copper, 0.1 p.p.m. added, 87.2% (range 83.3 to 90.7); 0.5 p.p.m. added, 92.2% (range 90.0 to 94.0); iron, 0.1 p.p.m. added, 97.5% (range 94.5 to 101.9); and nickel, 97.6% (range 95.4 to 100.8, for additions from 0.2 to 3.0 p.p.m.). The milk assays for copper and iron were corrected for recoveries at the 0.1 p.p.m. level, and the nickel analyses are presented as determined.

Because copper and iron were determined essentially simultaneously on the same aliquot of ash solution, the interference of copper and iron with each other at the 0.1 and 0.5 p.p.m. levels was determined. The average values obtained among five trials run in quadruplicate for copper are as follows: original milk, 0.032 p.p.m. copper; milk plus 0.096 p.p.m. iron, 0.031 p.p.m. copper; and milk plus 0.482 p.p.m. iron, 0.034 p.p.m. copper. Similar results were obtained for iron: original milk, 0.198 p.p.m. iron; milk plus 0.1 p.p.m. copper, 0.195 p.p.m. iron; and milk plus 0.5 p.p.m. copper, 0.194 p.p.m. iron. These data confirm those of Ventura and White *(38)* relative to the noninterference of copper and of iron on the determination of the other.

The copper, iron, and nickel contents of the raw milk (into the holding tank) and those of the first 30 gal. through the SS-WM system are shown in Table 1. The ranges of values obtained for the raw milks are: copper 0.022

TABLE 1

Copper, iron and nickel contents of milk before and after it passed through a system containing white metal fittings and stainless steel sanitary tubing

Date[a]	p.p.m. Cu[b, c, d]		p.p.m. Fe[b, c, d]		p.p.m. Ni[b, d]	
	Raw milk	Past. milk	Raw milk	Past. milk	Raw milk	Past. milk
4- 5-55	0.042	0.165	0.202	0.237	——	——
4-12-55	0.041	0.130	0.220	0.218	——	——
4-19-55	0.022	0.138	0.232	0.241	——	——
4-26-55	0.030	0.167	0.260	0.304	0.000	0.054
5- 3-55	0.030	0.084	0.236	0.216	0.000	0.045
5-10-55	0.044	0.150	0.257	0.257	0.000	0.022
5-17-55	0.040	0.092	0.222	0.223	——	——
5-24-55	0.033	0.118	0.275	0.204	——	——
5-31-55	0.040	0.147	0.224	0.252	——	——
6- 7-55	0.032	0.113	0.252	0.217	——	——
Av.	0.035	0.130	0.238	0.237	——	——

[a] Date milk received.

[b] Each value the average of triplicate determinations.

[c] Corrected for average recovery at the 0.1 p.p.m. level.

[d] Composite of first 30 gal. of milk through lines; pasteurization procedure: 72.8 ± 0.5° C., 16 sec., ca. 18 hr. after receipt.

to 0.042 p.p.m., iron 0.202 to 0.275 p.p.m., and nickel 0.000 p.p.m. The values for copper in raw milk are lower than those reported by Elvehjem *et al. (12)* (0.15 mg/l, ca. 0.145 p.p.m.), Davies *(9)* (0.15 to 0.65 p.p.m.), Krauss and Washburn *(26)* (0.14 to 0.17 p.p.m.), Dahlberg and Carpenter *(8)* (0.096 to 0.172 p.p.m.), McIlroy *(29)* (0.14 mg/l, ca. 0.135 p.p.m.), and Epple and Horrall *(13)* (0.21 to 0.24 p.p.m.); but they lie close to the ranges obtained by Conn *et al. (7)* (0.051 to 0.132 p.p.m.), Itzerott *(21)* (0.05 to 0.14 p.p.m.) and Munch-Petersen *(30)* (0.0553 p.p.m.). The increase in copper content as the milks passed through the SS-WM system averaged 0.095 p.p.m. (range 0.052 to 0.137 p.p.m.).

The iron contents of the raw milk samples are considerably higher (av. 0.238 p.p.m.) than those of copper; the increase during processing was negligible. Again the iron values are decidedly lower than those reported in raw milk by

Davies *(9, 10)* (1.50 to 2.40 p.p.m., and 1.18 to 2.52 p.p.m., respectively) and by Reis and Chakmakjian *(34)* (2.6 to 3.6 p.p.m.); however, they lie close to the range reported for raw milk by Krauss and Washburn *(26)* (0.36 to 0.51 p.p.m.), Dahlberg and Carpenter *(8)* (0.302 to 0.517 p.p.m.), and Itzerott *(21)* (0.30 to 0.77 p.p.m.). The values are higher than those reported for market milk by Holland and Ritchie *(19)* (1.2 to 1.5 p.p.m.) but agree closely with those reported for market milk by Johnston *(22)* and by Johnston *et al. (23)* (0.114 to 0.65, and 0.30 to 0.57 p.p.m., respectively).

No nickel was found in the raw milks, which is in agreement with the data of Archibald *(2)* for milks drawn directly into glass. The values for the processed samples ranged from 0.022 to 0.054 p.p.m. These quantities seemed sufficiently small to warrant discounting the nickel analyses after the third series, since Hunziker *(20)* and Henderson and Roadhouse *(18)* indicate that nickel is not particularly active as an oxidative catalyst. It must be recognized, however, that Guthrie *et al. (17)* indicated that nickel may cause oxidation in sweet milk.

The flavor scores of the milks are presented in Tables 2 and 3. Those in Table 2 designate maximum oxidative deterioration of the samples; the scores in Table 3 indicate the development and intensity of the cardboard, cappy and

TABLE 2

Flavor scores of milks during storage at ca. 4.4° C.

Date[a]	Type of milk	Flavor scores at following holding times after raw milk received (days)[b]					
		1	2	3	4	6	8
4- 5-55	Raw	10.0	9.8	8.5	8.0	6.7	D[d]
	Past.[c]	7.0	4.2	4.0	3.0	2.0	5.3
4-12-55	Raw	9.3	9.7	9.4	8.7	8.2	D
	Past.[c]	10.0	5.7	4.2	3.3	2.7	2.0
4-19-55	Raw	10.0	8.8	8.2	8.3	D	D
	Past.[c]	7.7	5.2	4.0	4.7	2.7	2.0
4-26-55	Raw	10.0	10.0	8.7	9.0	D	D
	Past.[c]	8.0	6.3	5.5	4.2	7.2	6.7
5- 3-55	Raw	9.5	10.0	9.7	8.3	D	D
	Past.[c]	10.0	6.7	3.8	3.7	4.7	5.7
5-10-55	Raw	10.0	9.3	9.7	9.7	9.3	D
	Past.[c]	9.7	6.7	5.5	6.3	6.0	5.3
5-17-55	Raw	9.7	10.0	10.0[e]	9.8[e]	D	D
	Past.[c]	10.0	8.5	8.0[e]	10.0[e]	8.7	D
5-24-55	Raw	10.0	10.0	10.0	10.0	10.0[f]	D
	Past.[c]	10.0	7.2	7.0	6.7	6.0[f]	9.5
5-31-55	Raw	10.0[e]	10.0	10.0	D	D	D
	Past.[c]	10.0[e]	8.3	7.8	6.8	7.5	7.3
6- 7-55	Raw	10.0	10.0	10.0	—	D	D
	Past.[c]	10.0	6.3	4.7	—	D	9.2
Av.	Raw	9.9	9.8	9.4	9.0	8.6	D
	Past.[c]	9.2	6.5	5.5	5.4	5.3	5.9

[a] Date milk received.

[b] Averages of scores of three judges unless otherwise noted.

[c] Composite of first 30 gal. of milk through lines; pasteurization procedure: 72.8 ± 0.5° C., 16 sec., ca. 18 hr. after receipt.

[d] All samples designated by *D* showed decomposition and were not included in averages.

[e] Averages of scores of two judges.

[f] Score of one judge.

TABLE 3

Flavor scores of milks during storage at ca. 4.4° C. for defects with numerical equivalents not less than 5

Date[a]	Type of milk	Flavor scores at following holding times after raw milk received (days)[b]					
		1	2	3	4	6	8
4- 5-55	Raw	10.0	9.8	8.5	8.0	6.7	D[d]
	Past.[c]	7.0	5.0[e]	—	—	—	7.5[e]
4-12-55	Raw	9.3	9.7	9.4	8.7	8.2	D
	Past.	10.0	5.7	5.0[e]	5.0[e]	—	—
4-19-55	Raw	10.0	8.8	8.2	8.3	D	D
	Past.	7.7	5.3	5.3	7.3	6.5[e]	7.0[e]
4-26-55	Raw	10.0	10.0	8.7	9.0	D	D
	Past.	8.0	6.3	5.7	6.2	7.2	6.7
5- 3-55	Raw	9.5	10.0	9.7	8.3	D	D
	Past.	10.0	6.7	5.0[e]	5.7	5.7	5.7
5-10-55	Raw	10.0	9.3	9.7	9.7	9.3	D
	Past.	9.7	6.7	5.7	6.3	6.0	5.3
5-17-55	Raw	9.7	10.0	10.0[e]	9.8[e]	D	D
	Past.	10.0	8.5	8.0[e]	10.0[e]	8.7	D
5-24-55	Raw	10.00	10.0	10.0	10.0	10.0[f]	D
	Past.	10.00	7.2	7.0	6.7	6.0[f]	9.5
5-31-55	Raw	10.0	10.0	10.0	D	D	D
	Past.	10.0	8.3	7.8	6.8	7.5	7.3
6- 7-55	Raw	10.0	10.0	10.0	—	D	D
	Past.	10.0	6.3	5.7	—	D	9.2
Av.	Raw	9.9	9.8	9.4	9.0	8.6	D
	Past.	9.2	6.6	6.1	6.8	6.8	7.3

[a] Date milk received.

[b] Averages of scores of three judges unless otherwise noted.

[c] Composite of first 30 gal. of milk through lines; pasteurization procedure: 72.8 ± 0.5° C., 16 sec., ca. 18 hr. after receipt.

[d] All samples designated by *D* showed decomposition and were not included in averages.

[e] Averages of scores of two judges.

[f] Score of one judge.

oxidized components of the over-all flavor. The raw milk samples developed defects as pronounced as "oxidized" in one case only (6th day, 4-5 sample); other defects were cardboard or cappy. The general trend of the HTST pasteurized samples was toward oxidized flavor development (Table 2); in some instances, slightly oxidized flavors were evident within 7 hours after processing. Metallic and tallowy flavors developed in several samples (4-5, 4-12, 4-19, 5-3). The variation of the scores and criticisms of the samples with storage time (Table 2) indicates that the intensity of the oxidized flavors reaches a maximum and then becomes less. This trend is likewise shown by the cardboard, cappy, and oxidized flavor components (Table 3).

Inasmuch as the increase in iron content was insignificant and that of nickel seemed unimportant, it may be that an increase of about 0.1 p.p.m. in the copper content is important in catalyzing oxidized flavor development during storage and that lesser amounts may be important in the production of cardboard and cappy flavors in milk produced under current handling conditions, which necessitate a greater age of the milk at the time of delivery. Pont's data *(33)* indicate that 0.1 p.p.m. Cu added to market milks generally (though not always) produced slight cardboard flavor in milk. Krukovsky and Guthrie *(28)* report oxidized flavor development by 0.1 p.p.m. added copper that was much more

rapid than that obtained by natural pick-up in these studies. These data suggest that Henderson and Roadhouse's hypothesis *(18)* that sufficient copper is not dissolved from SS-WM systems to cause oxidative deterioration in milk may not be valid with lower count milks held for longer times (after processing) before delivery.

These data likewise suggest that were the milk bottled directly, the first bottles off the line might develop oxidized flavor rapidly, with subsequent ones less and less rapidly. In fact, such situations have been reported by Tracy and Ruehe *(37)* and by Dahlberg and Carpenter *(8)* for milks contaminated from tinned copper, bronze pumps, and a nickel-copper-zinc alloy. The latter authors found that solution of copper and iron and acceleration of flavor defects occurred when milk at pasteurization temperature was passed through the lines; cold milk did not develop the defect. In the study reported in this paper the milk was cold as it passed through the system except at the HTST pasteurizer. This preliminary study indicates the need for more extensive studies with SS-WM systems.

The TBA values of the milks (Table 4) indicate *(a)* that there was little oxidation in raw milk, which agrees with the flavor scores in Tables 2 and 3; *(b)* that the presence of oxidation could be shown in microbiologically decomposed samples, when organoleptic evaluation could not be made; *(c)* that the

TABLE 4

2-Thiobarbituric acid values of milks during storage at ca. 4.4° C.

Date[a]	Type of milk	2-Thiobarbituric acid values at following holding times after raw milk received (days)[b]					
		1	2	3	4	6	8
4- 5-55	Raw	0.018	0.019	0.023	0.026	0.022	0.020[d]
	Past.[c]	0.030	0.053	0.063	0.064	0.060	0.053
4-12-55	Raw	0.013	0.014	0.015	0.012	0.013	0.017[d]
	Past.	0.015	0.035	0.053	0.049	0.057	0.051
4-19-55	Raw	0.022	0.046	0.022	0.022	0.024[d]	——
	Past.	0.024	0.066	0.058	0.063	0.058	——
4-26-55	Raw	0.022	0.020	0.025	0.022	0.022[d]	0.024[d]
	Past.	0.031	0.033	0.045	0.047	0.043	0.041
5- 3-55	Raw	0.023	0.022	0.022	0.023	0.023[d]	0.023[d]
	Past.	0.023	0.033	0.045	0.046	0.050	0.046
5-10-55	Raw	0.031	0.028	0.028	0.025	0.026	0.024[d]
	Past.	0.034	0.042	0.049	0.048	0.053	0.054
5-17-55	Raw	0.023	0.017	0.017	0.018	0.030[d]	0.030[d]
	Past.	0.023	0.022	0.025	0.027	0.062	0.029[d]
5-24-55	Raw	0.030	0.028	0.029	0.025	0.024[d]	0.025[d]
	Past.	0.031	0.046	0.047	0.042	0.044	0.024
5-31-55	Raw	0.017	0.023	0.022	0.023[d]	——	——
	Past.	0.022	0.029	0.032	0.033	——	——
6- 7-55	Raw	——	0.022	0.033	0.023	0.025[d]	0.016[d]
	Past.	——	0.034	0.053	0.050	0.028[d]	0.027
Av.	Raw	0.023	0.024	0.024	0.022	0.024	0.023[d]
	Past.	0.027	0.039	0.047	0.047	0.051	0.043

[a] Date milk received.

[b] Values expressed as optical density.

[c] Composite of first 30 gal. of milk through lines; pasteurization procedure: 72.8 ± 0.5° C., 16 sec., ca. 18 hr. after receipt.

[d] Samples which showed decomposition and were not scored organoleptically.

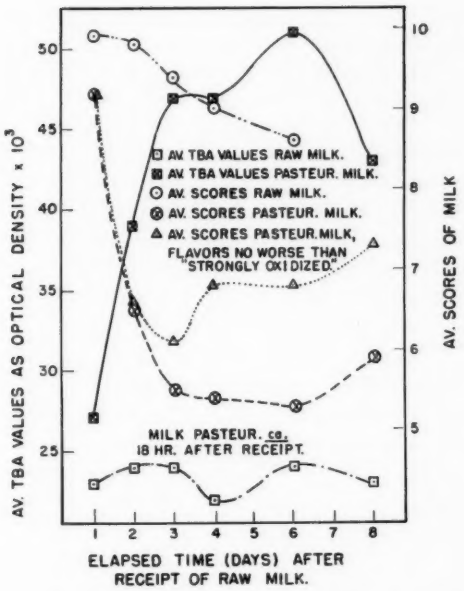
AV. TBA VALUES AS OPTICAL DENSITY x 10³
AV. SCORES OF MILK
AV. TBA VALUES RAW MILK.
AV. TBA VALUES PASTEUR. MILK.
AV. SCORES RAW MILK.
AV. SCORES PASTEUR. MILK.
AV. SCORES PASTEUR. MILK, FLAVORS NO WORSE THAN "STRONGLY OXIDIZED"
MILK PASTEUR. ca. 18 HR. AFTER RECEIPT.
ELAPSED TIME (DAYS) AFTER RECEIPT OF RAW MILK.

FIG. 2. Average flavor scores and thiobarbituric acid values of companion raw and pasteurized milks during an 8-day storage period.

spoilage organisms did not seem to produce compounds that increased the TBA color of the samples; and *(d)* that the HTST pasteurized samples showed an increase in TBA value followed by a decrease as storage time (at about 4.4° C.) increased, which again is in agreement with the organoleptic evaluation.

Two of the objectives in determining the TBA values were to attempt to establish: *(a)* whether or not the TBA values varied as the oxidized flavor of the milk varied and *(b)* whether the TBA values showed better correlation with the over-all oxidized flavor of the milk than it did with the cardboard, cappy, and oxidized component of the flavor, or vice versa. Figure 2 indicates that the TBA values and the milk scores vary inversely with each other. Based on the changes in the curvature of the plot of the average TBA values, the average over-all scores, and the average scores for the cardboard, cappy, and oxidized component, it would appear that the agreement was better between the TBA values and the cardboard to oxidized component than it was between the TBA values and the over-all oxidized flavor defects.

When this project was somewhat more than half completed, it was decided to pasteurize raw milk (from the drip sampler) in a laboratory pasteurizer that simulated spray vat pasteurization. It was considered that a comparison of the flavors, TBA values, and copper and iron contents of the companion LTLT and HTST pasteurized samples might indicate whether the increase in

copper in the HTST pasteurized milks might be the causative agent in the oxidation of these samples. Four such comparisons were made. The copper and iron values agreed well with those of the raw milk with one exception, in which the copper content increased but was lower than that of the HTST pasteurized sample. The copper may have entered the sample before pasteurization since no oxidized flavor developed *(4)*. No oxidation occurred in the LTLT pasteurized samples, and the TBA values were essentially constant. Except for a somewhat higher heated flavor in the LTLT than in the HTST pasteurized samples (which suggests somewhat higher sulfhydryl content), the comparisons suggest that the difference in oxidative susceptibility of the two sets of samples probably resulted from the copper dissolved from the SS-WM system.

During the last two series, the HTST pasteurized milks were separated into a "cream" and a "skimmilk" portion shortly after pasteurization, and on the third day after pasteurization with similar milk in the second from the last series. Flavor scores were obtained from the cream and skimmilk fractions and TBA values for the skimmilks. The cardboard to oxidized component of the over-all flavors was found in the skimmilk fractions, whereas the metallic and tallowy flavors were in the cream fraction. Moreover, the cardboard to oxidized component in the skimmilks increased in intensity and then decreased, as had been the case with the whole milks. These findings suggest that in these milks there were two distinct components to copper-induced oxidized flavor and that the cardboard to oxidized and the metallic components may readily be separated from each other by centrifuging the milk.

In connection with the two types of flavor noted above and the distribution of these flavors between the skimmilk and cream fractions, the literature is somewhat confusing. Frazier *(14)* stated that light-activated cardboard flavor developed in milk and cream from this milk but not in the equivalent skimmilk; Pont *(32)* considered that there were two types of light-activated flavor: cardboard and tallowy in the milk or its cream and scorched and gluey in the skimmilk, and he *(33)* considered that in copper-induced oxidation, cardboard flavor was produced in the skimmilk and oily-metallic flavors as a result of fat oxidation; Tracy and Ruehe *(37)* stated that sunlight causes a tallowy flavor accelerated by metals and a burnt flavor (in skimmilk) not accelerated by metals; Krukovsky *(27)* indicated that soapy-tallowy flavors occurred in the skimmilk fraction whereas metallic to fishy flavors were in the lipid fractions; and Guthrie *(16)* thought that metallic flavor was associated with the cream layer but not with the skimmilk layer.

The data resultant from this study are in agreement with the information cited above in that the flavor development resulting from copper-induced oxidation under commercial conditions is not a single flavor, but that at least two flavor types are involved, one more or less intimately associated with the cream fraction, the other with the skimmilk fraction. It is not within the province of this study to attempt to establish the precursors of the two flavors, although it would seem that the total lipids of the fat globule might be involved in the metallic to tallowy flavors in the cream layer.

SUMMARY AND CONCLUSIONS

Oxidation in both creamline and homogenized milk has been troublesome in Iowa, even during summer. This study was undertaken, therefore, to obtain refutation or confirmation of the hypothesis: The quantity of copper dissolved from stainless steel–white metal (SS-WM) systems could cause oxidation in milk with low-bacterial load, which is currently held longer times after pasteurization because of every other day or three times per week delivery.

Copper, iron, and nickel analyses, flavor scores, and thiobarbituric acid (TBA) values were obtained on raw and companion HTST milks once per week during 10 weeks (April 5 through June 7, 1955) so that both winter feed and pasture feeding regimes were included.

The ranges of metal contents of the raw milks were: copper, 0.022 to 0.042 p.p.m.; iron, 0.202 to 0.275 p.p.m.; and nickel, 0.000 p.p.m. The average increases in metal contents were: copper, 0.095 p.p.m.; iron, 0.0 p.p.m.; and nickel (3 series only), 0.04 p.p.m.

Generally all the milks which had passed through the SS-WM system oxidized readily. The flavor defects either increased during the entire storage period or tended to decrease on the sixth and/or eighth days. Comparisons (four series) of laboratory vat pasteurized and companion HTST pasteurized milks indicated that the increase in copper content was the likely cause of the oxidized flavor development of the milks which passed through the SS-WM system.

The over-all oxidized flavor seemed to have two components, one a cardboard to oxidized type which followed the skimmilk fraction, the other a metallic to tallowy type which followed the "cream" phase when the milks were separated in a laboratory centrifuge.

The flavor scores and TBA values varied inversely with each other. The data are interpreted as indicating closer correlation between the TBA values and the cardboard to oxidized component of the total oxidized flavor than with the over-all oxidative change.

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SOME PHYSICAL PROPERTIES OF MILK. III. EFFECTS OF HOMOGENIZATION PRESSURES ON THE VISCOSITY OF WHOLE MILK[1]

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The changes occurring in the viscosity of 4% fat milk homogenized at pressures of 15 to 3,500 p.s.i. were measured at temperatures ranging from 4° to 49° C. with an Ostwald viscometer. *Editor.*

The effects of homogenization upon the viscosity of pasteurized whole milk have been studied by many investigators *(1-5, 8, 10, 12-15)*. Wiegner *(16)* and Buglia *(2)* were among the first to report an increase in milk viscosity as a result of homogenization. In 1924, Evenson and Ferris *(5)* reported: "Homogenization of whole milk at 3,500 p.s.i. increases considerably the viscosity of milk. At 1,200 p.s.i. the increase in viscosity was to a small extent only." According to Bateman and Sharp *(1)*, who studied two samples of milk, the viscosity of whole milk was materially increased by homogenization. Later Doan *(4)*, Tretsven *(12)* and others reported similar results. However, Trout *et al. (14)* found that homogenization decreased the viscosity of pasteurized whole milk. Some investigators *(4, 12)* found the fat content of the milk to be an important factor, and homogenization temperature was reported by Doan *(4)* to be a factor of some importance. Differences in the temperatures at which viscosities were measured appear not to have been considered as a cause of conflicting results. The data on viscosity of milk were obtained with many types of viscometers, some of which lacked the precision to detect small variations in viscosity. The complicating effects of age or time of storage of milk have usually been ignored, and frequently precise temperature control of milk in the viscometer was lacking. After reviewing the literature pertaining to the viscosity of homogenized milk, Trout *(13)* stated: "Some differences of opinion seem to prevail among authorities as to the effect of homogenization upon the viscosity of milk. This is not surprising, considering the many possible temperature and pressure relationships which may exist in processing milk." In solutions as complex as milk, viscosity changes may be the result of many factors, some of which were discussed by Moore *et al. (9)* and by Hermans *(7)*. The purpose

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of this paper is to report the effects of homogenization pressures of 15 to 3,500 p.s.i. on the viscosity of whole, pasteurized milk at temperatures ranging from 4° to 49° C.

PROCEDURE

Mixed milk from 100 cows of six dairy breeds was used in these experiments. The milk contained 4% fat and 13% total solids. After pasteurization in a 200-gal. vat at 62° C. for 30 minutes, 30 gal. of milk was immediately divided into 20 samples. One unhomogenized sample was quickly cooled in a bath of ice water to 4° C. and stored until viscosities were measured. Ninteen samples were processed in homogenizers at 59° C. according to the following procedure: Each of nine samples was put through a laboratory homogenizer previously described *(10)* at one of the following pressures: 15, 25, 35, 50, 100, 175, 200, 250, or 300 p.s.i. Each of the remaining ten samples was processed in one stage of a commercial 125 gal. per hour homogenizer at one of the following pressures: 300, 500, 700, 1,000, 1,200, 1,500, 2,000, 2,500, 3,000, or 3,500, p.s.i. After homogenization, each sample was cooled as described above to 4° C. When that temperature was reached, the time was recorded as zero age. Measured viscosity values were adjusted to age 50 hours *(15)*.

Viscosities were determined with an Ostwald viscometer, modified as previously described *(15)*. The flow times of each milk sample were measured at two viscometer pressures because milk is a non-Newtonian liquid. The different pressures were obtained by placing the viscometer in a vertical and an inclined position. There was a three-fold increase in the flow time for water when the viscometer was inclined.

Statistical treatment of the viscosity data consisted of multiple linear regression analysis of the linear, quadratic, and cubic trends of viscosity with homogenization pressure. Two types of homogenizers, two positions of the viscometer, and nine temperatures at which viscosities were measured, were involved in this analysis.

RESULTS

As homogenization pressures were increased above 300 p.s.i. with a commercial sized homogenizer, there were obvious increases in the viscosity of the whole pasteurized milk at all temperatures at which viscosities were measured (Figure 1). The numerical increases in viscosity as a result of increasing homogenization pressures were greater when viscosities were measured at low temperatures. The relative increases, however, as determined by dividing the initial viscosity into the increase were not materially different at 49° C. from those at 4° C. Homogenization at pressures of 1,000, 1,500, 2,000, 3,000, and 3,500 p.s.i. increased viscosity as compared with the unhomogenized milk by respective averages of 7.1, 9.2, 11.9, 13.7, and 15.0%.

More complex effects on viscosity were found when the pasteurized whole milk was treated at lower pressures in the laboratory homogenizer. At all tem-

peratures, and when measured with the vertical viscometer, viscosities of the sample of milk that had been subjected to a homogenization pressure of 25 p.s.i. were lower than for the unhomogenized milk. Differences between the viscosities obtained with the vertical and inclined viscometer were greater after this pressure than for samples treated at any other homogenization pressure. Homogenization pressures, after which viscosities were least, varied when viscosities were measured with the inclined viscometer. At each temperature, and for some increase of homogenization pressure in the range 15 to 300 p.s.i., the viscosity decreased. This decrease in viscosity sometimes followed a previous increase at lower pressures. No reason is known for these more complex viscosity differences at low homogenization pressures. The fact that curves of viscosity versus homogenization pressure were usually nearly continuous at 300 p.s.i. indicates that similar homogenization effects were obtained from the commercial type and laboratory type of homogenizer.

TABLE 1

Statistical analysis[a] of factors affecting the viscosity of milk

Position of viscometer	Vert.			Incl.			Vert.			Incl.		
Homogenizer component of trend.	Lab. 15 to 300 p.s.i.						Coml. 300 to 3,500 p.s.i.					
	L	Q	C	L	Q	C	L	Q	C	L	Q	C
Temp. (°C)	Degree of significance[b]											
4	0	0	0	0	0	2	4	2	0	4	2	0
8	0	0	3	0	0	2	4	0	0	4	0	0
18	0	0	1	0	0	1	4	2	0	4	3	0
22.7	0	0	1	0	0	0	4	0	2	4	3	0
29	2	0	2	2	0	2	4	0	0	4	3	0
34	2	0	1	0	0	0	4	1	0	4	2	0
39	2	0	0	3	0	0	4	2	0	4	2	0
44	1	0	3	3	0	0	4	1	0	4	2	0
49	2	0	0	3	0	0	4	1	0	4	2	0

[a] Significance of linear, L, quadratic, Q, and cubic, C, components of trends of viscosity of milk with homogenization pressures increasing from 15 to 3,500 p.s.i. Tested at nine temperatures, using a laboratory or a commercial homogenizer, and using the viscometer both vertical and inclined enough to give a three-fold increase in flow time for water.

[b] 0 = F was above 10% level = N8; 1 = F in the 5 to 10% level = border line N8; 2 = F in the 1 to 5% level = *; 3 = F in the 0.1 to 1% level = **; 4 = F < 0.1% level = ***.

Statistical analysis (Table 1) indicates that the linear increase of viscosity with homogenization pressures over 300 p.s.i. was always significant at the 0.1% level (scored 4). This is the major over-all trend. The quadratic statistical trends, indicating some curvature in the general linear increase of viscosity with homogenization pressure, are somewhat obscured in Figure 1 by the logarithmic scale of pressures. However, they were significant (scored 2 or 3) in eight of the nine tests with the inclined viscometer. These trends were less pronounced for the vertical viscometer. The scores of 1 or 2 in six of the nine tests still appear to differ, however, from the scores of 0 in quadratic trends which were consistently found at pressures below 300 p.s.i. The existence of some significant cubic trends indicated a tendency for a curve to be concave

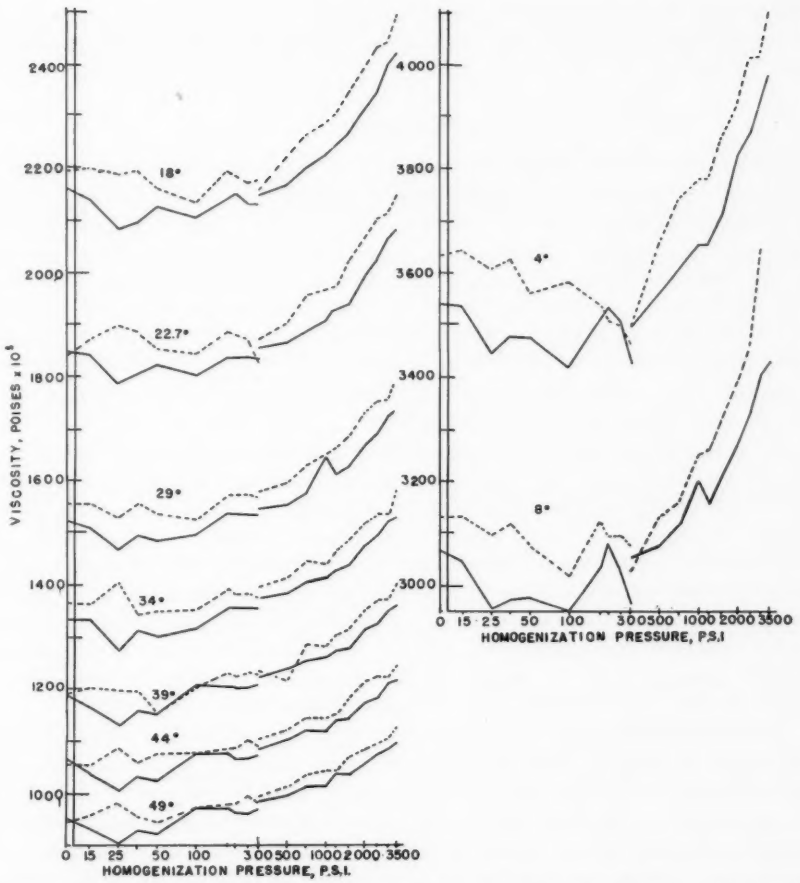

FIG. 1. Effects of homogenization pressure on viscosities of milk. All viscosities adjusted to age 50 hours, and measured with viscometer vertical (———) or inclined (– – – – –). Breaks at 300 p.s.i. are at change from laboratory to commercial homogenizer.

upward over one pressure range but concave downward over another range. At pressures over 300 p.s.i. such cubic trends were not significant even at a 10% level (scored 0) in all tests with the inclined viscometer and in eight of the nine tests with the vertical viscometer.

When milk was homogenized at pressures below 300 p.s.i., the only significant viscosity-pressure trends at temperatures below 23° C. were cubic. At higher temperatures the linear trend was more consistent with the vertical viscometer (scored 1 or 2) but was usually more pronounced (scored 0, 2, 3) with the inclined viscometer. Quadratic trends at pressures below 300 p.s.i. continued to score 0 at the higher temperatures. The significant cubic statistical

trends at some temperatures usually indicated a tendency for viscosity to increase at pressures near 300 p.s.i. after a decrease beginning at pressures near 15 p.s.i.

DISCUSSION

The increase in the viscosity of pasteurized whole milk with homogenization pressure, reported here, substantiates and extends the range of some studies previously reported *(1, 5)*. The lower homogenization pressures used in this study are not enough to significantly disintegrate fat globules. Effects of suspended inert particles on viscosity depend on the total volume of particles rather than on the size of individual particles. The exact causes of changes in the viscosity of milk as a result of homogenization are therefore obscure. They may be entirely independent of the changes in fat particle size used to define homogenization. They may include indirect effects of decreased particle size on the volume and composition of globule membranes.

Increases in the viscosity of colloids are sometimes ascribed *(a)* to changes in particle shape (e.g., uncoiling of chains or unfolding of surfaces in protein particles); *(b)* to transfer of material from the continuous phase to the disperse phase (e.g., the complexing of proteins with water, sugar, fat, or enzymes); or *(c)* to electroviscous effects. Electroviscous effects may involve electric charges or zwitterions on the surface of protein particles and possibly also charges buried in the folds or coils of the long chains of a globular protein. Effects similar to electroviscous may also involve Van der Waal's type of interaction between particles *(11)* or degrees of hydrogen bonding (e.g., hydration) not strong enough to be recognized as stable complexes *(6)*.

Effects of temperature on viscosity may more clearly indicate causes of these differences in viscosity. The precision of several theoretical and empirical equations relating the temperature and viscosity of milk is being studied. Effects of such factors as previous heat treatment, mineral content or nature of mineral combinations, amount of numerous minor organic constituents, as well as concentration of major constituents, are a wide field for numerous studies. Differences in viscosity not only directly affect the stability and attractiveness of some fluid milk products but also may be important in the processing of milk products for use in other foods.

SUMMARY

Data on effects of homogenization pressures from 15 to 3,500 p.s.i. on the viscosity of whole pasteurized milk at temperatures from 4° to 49° C. are reported. As homogenization pressures were increased above 300 p.s.i. there were highly significant linear increases in viscosity when measured at 9 temperatures. Pressures of 1,000, 1,500, 2,000, 3,000, and 3,500 p.s.i. increased viscosity as compared with the unhomogenized milk by respective averages of 7.1, 9.2, 11.9, 13.7, and 15.0%. Quadratic trends were also present. At pressures below 300 p.s.i. and temperatures above 23° C., linear trends were usually significant. At

lower temperatures there were no significant linear or quadratic trends. Cubic trends sometimes indicated an increase in viscosity at pressures near 300 p.s.i. after a decrease beginning at pressures near 15 p.s.i.

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THE SPECIFICITY OF MILK LIPASE. I. DETERMINATION OF THE LIPOLYTIC ACTIVITY IN MILK TOWARD MILK FAT AND SIMPLER ESTERS[1, 2]

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In the study of the lipolytic activity in milk toward milk fat, standard methods of procedure are necessary. This study reports the results of the optimum conditions for the activity of milk lipase toward a variety of esters. The pH optima for milk fat and esters studied are given. *Editor.*

The choice of proper substrates for lipase determination has been a subject of considerable controversy in biochemical and dairy literature *(2, 7, 14, 15, 27)*. Disagreement in the choice of substrates may stem from the conflicting results obtained in studies of the specificity of this group of enzymes *(2, 7)*. In regard to milk, part of the confusion may be attributed to the arbitrary conditions chosen for the development of lipolysis.

To avoid some of the difficulties arising from the use of milk fat as an emulsion, several investigators have proposed to measure the lipase activity of milk with simpler esters. Tributryrin has been one of the most common substrates used *(1, 17, 18, 20, 21, 26)*. Chromogenic esters have been used to permit the application of sensitive colorimetric methods *(9, 13)*. Tween-20, a soluble polyoxyethylene ester of lauric acid and sorbitan, has been commonly used in systems other than milk *(4, 5, 11)*. However, since these esters differ greatly from the natural substrates of lipase, their use for lipase determination has been questioned *(7, 14, 15, 19, 22, 27)*.

The lipolytic activity of milk toward various natural fats was studied by Gould *(12)*. Kelly *(16)* compared the hydrolysis of different simpler esters and milk fat, but the optimum conditions for hydrolysis were not determined. Dunkley and Smith *(8)* found a correlation between the tributyrin and milk fat hydrolyses in the milk from individual cows and a similarity in the pH-activity curves for these two activities at 10° C. These results were considered by the authors to provide justification for the use of tributyrinase determinations as a measure of

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[2] This study was supported in part by funds from the California Dairy Industry Advisory Board.

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lipase in milk. However, the implications of their study are difficult to interpret because, although the conditions used for the tributyrin hydrolysis were optimal, not all of those used for the milk fat hydrolysis were. In addition, the reliability of the method used by these authors for the determination of free fatty acids liberated from milk fat was not evaluated.

The optimum conditions for the hydrolysis of tributyrin in milk have been well standardized *(17, 20, 21, 26)*. Unfortunately, similar efforts have not been made to standardize methods of determining the lipolytic activity in milk toward milk fat. The optimum pH has been reported for lipolysis in cream samples as 8.4 to 8.6 at 3-4° C. *(23)*, and for the lipolysis of milk fat homogenized in rennet whey as between 8 and 9 at 35-40° F. *(12)*. However, the reaction mixtures in these studies were not buffered, and the pH therefore varied during the reaction period. Dunkley and Smith *(8)* found an optimum pH of 9.5 at 10° C. for the lipolysis of milk fat homogenized in skimmilk and buffered with 0.1 *M* borate. In all of the above studies the substrate concentration and times and temperatures of reaction were chosen arbitrarily. Yet these factors greatly influence the optimal conditions for enzyme action. Schwartz *et al. (25)* have reported a study of factors affecting the lipase activity by employing freeze-dried skimmilk as the lipase source and milk fat as the substrate, but the results have not been published.

The specificity of milk lipase was studied in this series of papers on the basis of the relative lipolytic activity in milk toward Tween-20, methyl butyrate, tributyrin, and milk fat. These esters were chosen to vary widely in solubility and susceptibility to lipase. The relative lipolytic activity in milk toward such a variety of esters can only be compared at optimum conditions for activity. This paper presents a study of the optimum conditions for the activity in milk toward these esters, by using extraction-titration methods for the determination of free fatty acids.

EXPERIMENTAL PROCEDURE

Materials. The enzyme source consisted of skimmilk prepared by centrifugation from freshly drawn raw mixed milk. The skimmilk was used as such or lyophilized. The skimmilk powder was resuspended in water or buffer before use with a Servall Omni-mixer[4] at low speed.

Stock solutions of Tween-20[5] and methyl butyrate[6] and stock emulsion of tributyrin[6] were prepared each time before use (to reduce to a minimum the development of fatty acids upon standing). Free fatty acids were removed from Tween-20 by passing a 20% solution (by weight) through an ion exchange column of Amberlite IRA 400,[7] activated with 0.1 *N* NaOH by the method of Borgström *(6)*. The tributyrin emulsion (12.5%) was prepared by emulsifying with the

[4] This is a stainless steel blendor actuated by a 15,000 r.p.m. motor, supplied by Servall, Inc., New York.

[5] Obtained from Atlas Powder Co., Wilmington, Delaware.

[6] Obtained from Eastman Kodak Co., Rochester, New York.

[7] Donated by Rohm and Haas Co., Philadelphia, Pennsylvania.

Omni-mixer (at low speed) a mixture of 2.0 g. tributyrin, 5.0 ml. 1 *M* borate (pH 7.5), 1 g. skimmilk powder (Starlac), and 8.0 ml. distilled water. Pasteurized homogenized cream containing 38 to 40% fat was used as the source of milk fat. This cream was obtained from pasteurized milk (holding) and homogenized at 2500 p.s.i. (2nd stage = 500 p.s.i.) at 40° C. The homogenized cream was steamed for 12 minutes in the presence of 1% toluene in lots of 50 ml. in open 125-ml. Erlenmeyer flasks under a slight positive pressure. The cream thus treated could be kept at 0-5° C. in tightly stoppered flasks for more than a month without any bacterial growth. Toluene had no deleterious effect on the lipase activity.

Methods. The assay method consisted of reacting the buffered reaction mixture in 50-ml. Erlenmeyer flasks in a Warburg constant temperature bath[8] at 37.0° C. for appropriate time intervals with constant horizontal shaking (about 150 cycles per minute). The procedure was carried out as follows. The substrate was measured into the reaction vessels, followed by the buffer and a predetermined volume of 0.5 *N* or 1 *N* NaOH and distilled water to a given volume. After temperature equilibration in the bath for 10 minutes, the enzyme material was added and the contents were well mixed. The free fatty acids were determined in aliquot samples of reaction mixture taken at zero time and at the end of the reaction period. The reaction was stopped in these samples by addition to either alcohol (for Tween-20 and milk fat) or phosphoric acid (for methyl butyrate and tributyrin) in the centrifuge tubes to permit subsequent extraction of free fatty acids.

The optimum conditions found and adopted for determination of the lipolytic activity of milk were as follows: enzyme — 8% lyophilized skimmilk or 40% fresh skimmilk; substrates — 4% Tween-20, 4% tributyrin, 0.07 *M* methyl butyrate, 15% milk fat; buffer — 0.1 *M* borate; pH — 8.8 for Tween-20 and tributyrin, 8.0 for methyl butyrate and 8.8-9.0 for milk fat; reaction time — 1 hour for Tween-20, methyl butyrate and milk fat, 15 minutes for tributyrin; temperature — 37.0° C. The composition of the reaction mixtures as routinely used in this study was as follows: Tween-20: 2.50 ml. 1 *M* borate (pH 7.5) + 5.0 ml. 20% Tween-20 + 10.0 ml. skimmilk + 1 *N* NaOH to pH 8.8. + distilled water to 25.0 ml. Methyl butyrate: 14.0 ml. 0.15 *M* methyl butyrate in borate buffer (17.85 ml. 1 *M* borate per 100 ml. solution) + 10.0 ml. skimmilk + 0.5 *N* NaOH to pH 8.0 + distilled water to 25.0 ml. Tributyrin: 4.0 ml. 12.5 % buffered tributyrin emulsion + 5.0 ml. skimmilk + 1 *N* NaOH to pH 8.8. + distilled water to 12.5 ml. Milk fat : 1.25 ml. 1 *M* borate + 4.7 g. 40% homogenized cream + 5.0 ml. skimmilk + 1 *N* NaOH to pH 8.8-9.0 + distilled water to 12.5 ml.

Controls were run for possible nonenzymatic hydrolysis (with pasteurized skimmilk) or endogenous hydrolysis (in the absence of substrate) at the optimum pH. No hydrolysis could be detected under these conditions for any of the substrates. The possible contribution of any bacterial action also was checked. No

[8] The manometer supports (without the manometers) were fitted with special clamps to firmly hold the reaction vessels immersed about 3 cm. below the water surface.

increase in the plate counts occurred during the first two hours of reaction (milk fat hydrolysis).

The "activity unit" as used in this study is defined as milliliters of 0.01 *N* NaOH (required to neutralize the fatty acids liberated) per minute (of reaction time). After standardization of the procedure, the activity units were converted into "enzyme units" (or "lipase units" for milk fat hydrolysis), one enzyme unit being equal, by definition, to the amount of enzyme that produces one activity unit.

A modification of the extraction-titration method of Frankel and Tarassuk *(10)* was used for determinations of lauric acid from Tween-20 hydrolysis and fatty acids from milk fat hydrolysis. In order to obtain maximum recovery of these fatty acids, it was necessary to acidify the alkaline reaction mixtures to pH 6 prior to ether extraction to repress the ionization of the free fatty acids and thus increase their solubility in organic solvents. Butyric acid from methyl butyrate and tributyrin hydrolyses was determined by the method of Smith *et al. (26)* with minor modifications. The extraction-titration procedures were performed as follows:

1. *Lauric acid and fatty acids from milk fat:* A 5.0-ml. aliquot of Tween-20 hydrolyzate or a 2.0-ml. aliquot of milk fat hydrolyzate was shaken vigorously with 5 ml. of neutralized 95% ethanol in a 50-ml. centrifuge tube for 30 seconds. One drop of 0.04% methyl red was added to the mixture, which was then titrated with 0.5 *N* HCl to a distinct pink color (pH 6). Fifteen ml. of ether mixture (5:1 and 2:3 ethyl ether–petroleum ether by vol. for Tween-20 and milk fat, respectively) was added and the mixture was shaken another 30 seconds. After a centrifuging period of 3 minutes at 1500 r.p.m., the top ether layer was removed with a siphoning device[9] and added to a 125-ml. Erlenmeyer flask containing 10 ml. neutralized 95% ethanol. The remaining aqueous layer was extracted again with 10 ml. ether mixture. The alcohol-ether mixture was then titrated with 0.020 *N* alcoholic KOH (95% ethanol) to the phenol red end-point (10 drops 0.05% alcoholic phenol red), with a 2-ml. microburette graduated to 0.010 ml. A blank value was taken on the hydrolyzates at 0 time to take care of the titer due to reagents and aqueous phase.

The recovery of lauric acid by the above extraction-titration procedure was 95%.

2. *Butyric acid:* A 5.0-ml. aliquot of methyl butyrate hydrolyzate or 2.0-ml. aliquot of tributyrin hydrolyzate was treated with 5 ml. 1 *M* phosphoric acid by inverting the tube twice. This mixture was extracted only once with 20.0 ml. ethyl ether by shaking the tube vigorously for 5 seconds. After centrifuging at 1500 r.p.m. for 3 minutes, a 10.0-ml. aliquot was pipetted from the supernatant ether layer and mixed into a 125-ml. Erlenmeyer flask with 10 ml. neutralized

[9] Similar to that described by Hillig (*J. Assoc. Offic. Agr. Chemists*, 30: 575. 1947; see Footnote 1 on p. 580).

95% ethanol. Titration of this ether-alcohol mixture was carried out as above and the titration reading was multiplied by two.

The precision of the method was evaluated by carrying out 10 measurements at two levels of free fatty acids. The error of the method was 1 to 2% and 3 to 5% with titers of 3.50 and 1.00 ml. of 0.020 *N* KOH, respectively.

RESULTS

Relation of activity to pH. Preliminary experiments were carried out to determine the effect of borate and veronal buffers on the activity of milk toward milk fat. Reactions carried out in the presence of buffers (0.025 *M* to 0.1 *M*) were compared with nonbuffered reactions in which pH was kept constant by continuous titration with standard alkali. The veronal buffer was not satisfactory because it gave high zero-time readings with the extraction-titration method used, and the end-point with phenol red was indistinct. With borate, the activity in the buffered reaction mixture corresponded to that in the nonbuffered reaction mixture. Therefore, borate at the concentrations used does not inhibit the lipase action of milk. A concentration of 0.1 *M* borate was sufficient to maintain the pH of the reaction mixtures within 0.1 unit of the optimum after 1 hour of reaction.

The effect of pH on activity was determined for the four substrates at optimal substrate concentration and temperature. The results presented in Figures 1 and 2 show pH optima ranging from 8.8 to 9.1 for milk fat hydrolysis, 8.8 for

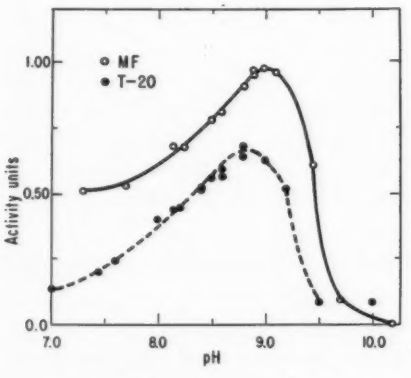

FIG. 1. Relation between the hydrolysis of milk fat (MF) and Tween-20 (T-20) in milk and pH. Activity units: per 100 ml. reaction mixture. (Enzyme source: 5% lyophilized skimmilk; substrate: 15% MF, 4% T-20; buffer: 0.1 *M* borate; reaction time: 1 hr.; temperature: 37° C.)

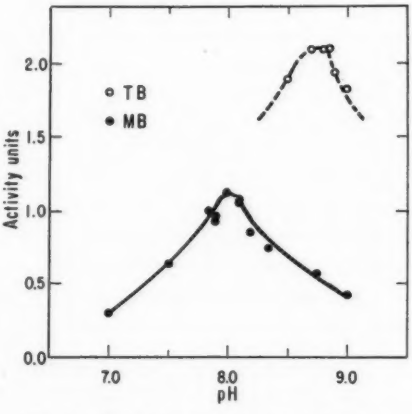

FIG. 2. Relation between the hydrolysis of tributyrin (TB) and methyl butyrate (MB) in milk and pH. Activity units: per 100 ml. reaction mixture. (Enzyme source: 5% lyophilized skimmilk; substrate: 4% TB, 0.07 *M* MB; buffer: 0.1 *M* borate; reaction time: 15 min. for TB, 1 hr. for MB; temperature: 37° C.)

Tween-20 hydrolysis, 8.0 for methyl butyrate hydrolysis, and 8.7 to 8.9 for the tributyrin hydrolysis in milk. The data for the tributyrin hydrolysis agree with those in the literature *(17, 20, 26)*.

Relation of activity to substrate concentration. Determination of the substrate concentration that will not become a limiting factor in the lipolytic activity of milk is of particular importance in specificity studies. It is only in this range of substrate concentration that the relative lipolytic activity in milk toward different substrates can be related to differences in specificity. The use of tributyrin and milk fat as emulsions in water involves an additional consideration — the active surface of the substrate particles. It was assumed in this study, as did Schönheyder and Volqvartz *(24)*, that the triglycerides during constant shaking are emulsified in particles fine enough not to limit the action of the enzyme(s) at the lipid-aqueous interface.

For milk fat hydrolysis, the effect of homogenizing cream (used as substrate) at various pressures was studied. The results (Table 1) show that significant

TABLE 1

Effect of homogenizing cream at different pressures on the lipolytic activity of raw skimmilk

Homogenizing pressure	Titration value
(p.s.i.)	*(ml. 0.020 N KOH)*
Control (nonhomogenized)	0.77
500	0.98
1000	0.98
1500	1.01
2000	1.04
2500	1.07
3000	1.13

hydrolysis took place with the nonhomogenized control cream. On the other hand, the difference between the creams homogenized at 500 and 3,000 p.s.i. was within experimental error. It should be noted that partial oiling off occurred in the reaction mixture containing nonhomogenized cream. This destabilization of the emulsion is probably due to the shaking at 37° C. and the alkaline conditions of the reaction. To obtain reproducible emulsions, cream homogenized at 2,500 p.s.i. was chosen for the assays.

Since changes in the concentration of fat in cream will affect the recovery of fatty acids by the extraction-titration method used *(10)*, the size of the aliquots taken for free fatty acid determination in this case was varied so that the amount of fat was kept constant — corresponding to 2.0 g. of reaction mixture containing 15% fat. This precaution was not necessary for the other substrates.

The effect of substrate concentration on the lipolytic activity in skimmilk toward the different substrates is represented in Figures 3 and 4. The substrate concentration at which the lipolytic activity in a given amount of skimmilk reaches a maximum is 15% for milk fat, 4% for Tween-20, and 0.07 *M* for methyl butyrate. These substrate concentrations were used in the assay procedures for equivalent amounts of skimmilk used as enzyme source. The constant hydrolysis

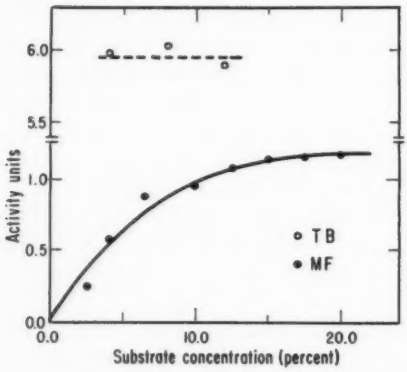

FIG. 3. Relation between the hydrolysis of milk fat (MF) and tributyrin (TB) in milk and substrate concentration. Activity units: per 100 ml. reaction mixture. (Enzyme source: 8% buffered lyophilized skimmilk; buffer: 0.1 *M* borate; pH: 8.8; reaction time: 1 hr. for MF, 15 min. for TB; temperature: 37° C.)

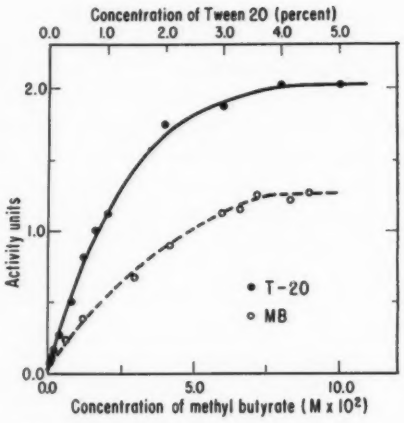

FIG. 4. Relation between the hydrolysis of Tween-20 (T-20) and methyl butyrate (MB) in milk and substrate concentration. Activity units: per 100 ml. reaction mixture. (Enzyme source: 8% buffered lyophilized skimmilk; buffer: 0.1 *M* borate; pH: 8.8 for T-20, 8.0 for MB; reaction time: 1 hr.; temperature: 37° C.)

obtained for tributyrin between 4 and 12% agrees with the maximum found by Smith *et al. (26)*. A concentration of 4% tributyrin was used in this study.

Velocity of hydrolysis. It is desirable for assay purposes to choose short reaction times during which zero order kinetics prevail if the substrate concentration is sufficient to saturate the enzyme. Under these conditions one can express activity values directly in concentration (of fatty acids) per reaction time. For these experiments the assays were carried out at optimal conditions in large reaction vessels, taking aliquot samples at regular time intervals for free fatty acid determinations.

The data in Figure 5 show that the reaction periods during which the hydrolyses are proportional with time are 180, 60, and 90 minutes for milk fat, methyl butyrate, and Tween-20, respectively. For the tributyrin hydrolysis in milk, zero order kinetics prevailed during the first 20 minutes only. On the basis of these results the reaction times chosen for the assays were 1 hour for the hydrolysis of milk fat, methyl butyrate, and Tween-20 and 15 minutes for the hydrolysis of tributyrin in milk.

Relation of activity to skimmilk concentration. The activities obtained with various concentrations of skimmilk in the reaction mixtures were studied to determine if the enzyme concentration was the limiting factor in the assays. The rate of hydrolysis produced by different weights of lyophilized skimmilk on Tween-20 and methyl butyrate was determined under optimal conditions. The results in Figure 6 show that this relation was linear at all enzyme concentrations

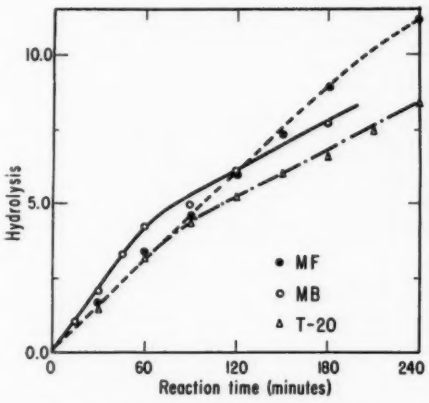

FIG. 5. Velocity of hydrolysis of milk fat (MF), methyl butyrate (MB) and Tween-20 (T-20) in milk. Hydrolysis: ml. 0.01 *N* KOH per 5 ml. reaction mixture. (Enzyme source: 5% buffered lyophilized skimmilk; substrate: 15% MF, 0.07 *M* MB, 4% T-20; buffer: 0.1 *M* borate; pH: 8.9 for MF, 8.0 for MB, 8.8 for T-20; temperature: 37° C.)

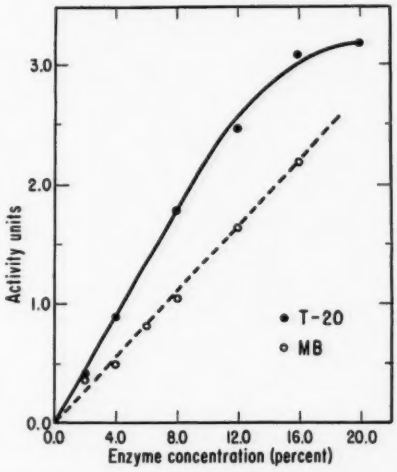

FIG. 6. Relation between the hydrolysis of Tween-20 (T-20) and methyl butyrate (MB) in milk and enzyme concentration. Activity units: per 100 ml. reaction mixture. (Enzyme source: lyophilized skimmilk; substrates: 4% T-20, 0.07 *M* MB; buffer: 0.1 *M* borate; pH: 8.8 for T-20, 8.0 for MB; reaction time: 1 hr.; temperature: 37° C.)

used for the hydrolysis of methyl butyrate, and linear up to 8 to 12% skimmilk powder for the hydrolysis of Tween-20. It should be noted here that the reaction mixture became quite viscous when the amount of skimmilk powder exceeded 8%. On the basis of these results the "activity unit" for Tween-20 and methyl butyrate hydrolyses in a sample was taken (by definition) as equal to the "enzyme unit." The same was done for the hydrolysis of tributyrin in milk, which has been found to be proportional to the enzyme concentration in the reaction mixture *(26)*. Precautions were taken in these cases to regulate the amount of milk used in the assays to be within the range of activity where it is proportional to enzyme concentration.

The relation between milk fat hydrolysis and skimmilk concentration was not linear. Therefore, in this case a standard curve had to be established to convert activity units into enzyme or lipase units. This curve was set up with fresh skimmilk from three lots of mixed milk and with a casein gel suspension obtained by supercentrifugation with a Sharples centrifuge. Different concentrations were obtained by dilution with pasteurized skimmilk or pasteurized casein suspension. The relation between activity units and concentration of enzyme sources is presented in Figure 7. The method of Anson *(3)* for the standardization of assay procedures was used.

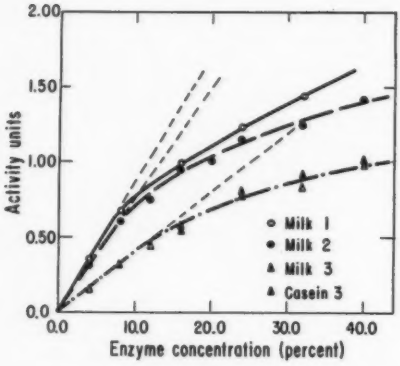

FIG. 7. Relation between the hydrolysis of milk fat in different milks and casein and enzyme concentration. Activity units: per 100 ml. reaction mixture. (Enzyme source: fresh skimmilk and casein gel suspensions; substrate: 15% milk fat; buffer: 0.1 *M* borate; pH: 8.9; reaction time: 1 hr.; temperature: 37° C.)

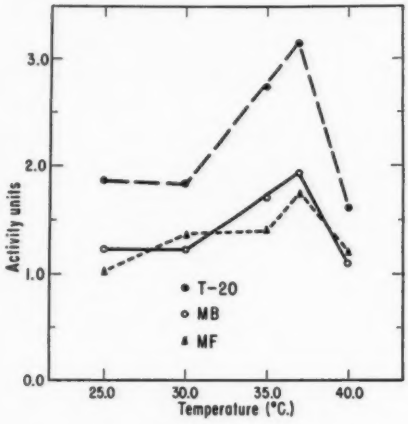

FIG. 8. Relation between the hydrolysis of Tween-20 (T-20), methyl butyrate (MB) and milk fat (MF) in milk and temperature. Activity units: per 100 ml. reaction mixture. (Enzyme source: 8% lyophilized skimmilk; substrates: 4% T-20, 0.07 *M* MB, 15% MF; buffer: 0.1 *M* borate; pH: 8.8 for T-20, 8.0 for MB, 8.9 for MF; reaction time: 1 hr.)

Relation of activity to temperature. The rates of hydrolysis of Tween-20, methyl butyrate, and milk fat were determined at temperatures varying from 25° to 40° C. The results (Figure 8) show that the maximum rate of hydrolysis for all substrates occurs at 37° C. The same temperature optimum has been obtained for the tributyrinase hydrolysis in milk *(26)*. It should be noted here that partial churning or oiling-off took place in milk fat hydrolysis at temperatures below 35° C. This may have caused the leveling off of this activity between 30 and 35° C.

DISCUSSION

A large part of the present confusion in the literature on milk lipase may be related to the arbitrary methods used for lipase determinations. Several investigators have tried to relate lipase concentration to the development of lipolysis at certain arbitrarily chosen conditions. Much of the work on milk lipase was done with milk or cream, giving little consideration to the concentration of available substrate. Exclusion of bacterial action has been achieved by using chemical preservatives, saturating the substrate with sugar, or carrying out lipolysis at low temperatures. All these procedures tend to limit lipase action and therefore necessitate long periods of incubation to obtain appreciable activity. With such long reaction times, complications may arise from mixed order reaction

kinetics, accumulation of inhibiting reaction products, varying substrate concentration, and denaturation effects from aging.

By a systematic evaluation of the optimum conditions for the lipolytic activity of milk, it was possible in this study to obtain measurable activities within relatively short reaction times during which zero order kinetics prevailed. This permitted the determination of initial velocities, which will be used in some of the kinetic data in the following paper of this series. Such optimum conditions are essential in specificity studies of milk lipase based on the relative activity toward esters varying widely in structure.

SUMMARY

A study of the optimum conditions for the activity of milk toward different esters was made. This will provide the basis for the assay methods used in further specificity studies. The reactions were carried out with constant shaking. Under these conditions, appreciable hydrolysis of milk fat occurred with nonhomogenized cream. The emulsion was more stable, however, with homogenized creams. Cream homogenized at 2,500 p.s.i. was therefore used for the milk fat hydrolysis. The pH optima for the milk fat, Tween-20, and methyl butyrate hydrolyses were 8.8-9.1, 8.8, and 8.0, respectively. The pH optimum of 8.7-8.9 for the tributyrinase activity of milk was confirmed.

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THE SPECIFICITY OF MILK LIPASE. II. KINETICS, AND RELATIVE LIPOLYTIC ACTIVITY IN DIFFERENT MILKS[1, 2]

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The lipolytic activity in milk toward Tween-20, methyl butyrate, tributyrin, and milk fat was determined. Variations in the activity observed may be considered as an indication of the multiplicity of lipolytic enzymes present in milk. *Editor.*

The first paper of this series *(3)* described methods developed to determine the relative lipolytic activity in milk toward Tween-20, methyl butyrate, tributyrin, and milk fat. The present paper deals with an evaluation of kinetic data and a survey of the lipolytic activity in different milks, i.e., activity toward Tween-20, methyl butyrate, tributyrin, and milk fat, as evidences for the multiplicity of lipases in milk.

The question of whether more than one enzyme is present in a system which can hydrolyze a given substrate can be best solved by isolation and study of the pure enzyme(s). This is, of course, a laborious procedure, and as yet no purified lipase preparation has been obtained from milk. However, other approaches to this problem are possible without isolation of enzyme(s).

On the basis of the Michaelis-Menten theory some useful information can be obtained as to whether more than one enzyme is involved in a reaction system. The action of the enzyme preparation can be studied on two substrates at varying concentrations to determine whether or not the substrates compete for the active group(s) of the enzymes(s) *(7, 10)*. In another approach, one substrate is used in a wide range of concentrations, and the kinetic data treated according to one of the linear forms of the Michaelis-Menten equation: $Vm = v + v/S$. Km *(4, 5)*. For a reaction involving one enzyme, a plot of v (initial velocity) against v/S (where S is the substrate concentration) will give a straight line with slope equal to Km, the Michaelis-Menten constant. If more than one enzyme is involved in the system, this relation will deviate from linearity and each enzyme would contribute to the curve in proportion to its affinity toward the substrate.

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[2] This study was supported in part by funds from the California Dairy Industry Advisory Board.

[3] Present address: Northern Utilization Research Branch, USDA, Peoria, Ill.

A different way to evaluate the possible heterogeneity of an enzyme system — one used in this study — can be based on the relative activity in different milks toward different substrates. Whether the relative lipolytic activity remains constant or varies with the respective milk is the basis for the identification of one or different enzymes in the systems. Other approaches to this problem will be taken up in the following papers of this series.

Schwartz *et al. (13)* reported the presence of three different lipases in milk. Their evidence[4] was based on the multiple optima obtained in the pH-activity curve for milk fat hydrolysis, the nonlinearity of v against v/S plotted according to Hofstee's treatment *(5)*, and the change of the shape of pH-activity curve by formaldehyde inhibition. In a different investigation, Albrecht and Jaynes *(1)* presented evidence for the presence of two general enzyme systems in milk responsible for the hydrolysis of tributyrin and ethyl butyrate on the basis of two pH optima at pH 5.4 and 6.3. Within each general system two enzymes were indicated on the basis of formaldehyde sensitivity and substrate specificity. Interpretation of such evidences is very difficult because of the numerous factors affecting the relationships between enzyme activity, pH, and substrate concentrations. It is well recognized that the pH optimum, like the temperature optimum, is not a true characteristic of an enzyme *(9)* and therefore could not be used to identify enzyme(s). Comparing pH activity curves for tributyrin and milk fat hydrolyses to obtain some basis for the determination of lipase by measuring tributyrinase *(2)* may be questioned on the same grounds.

EXPERIMENTAL PROCEDURE

The skimmilk was obtained from freshly drawn mixed milk. For the kinetic experiments, it was lyophilized and reconstituted in borate buffer before use. For the survey of the relative lipolytic activity in different mixed milks, the milk samples were cooled to 0° C. immediately after milking and assayed within approximately 2 hours after milking. The lipolytic activity in milk toward Tween-20, methyl butyrate, tributyrin, and milk fat was determined by the methods developed in the first paper of this series *(3)*.

RESULTS AND DISCUSSION

The relation of activity to substrate concentration was determined for the hydrolysis of milk fat, Tween-20, and methyl butyrate, as described in the previous paper *(3)*. The kinetic data were plotted according to some linear forms of the Michaelis-Menten equation *(8)* to estimate the Michaelis-Menten constant *(Km)* and the maximum velocity *(Vm)*. Different relations between v (initial velocity) and S (substrate concentration) are presented in Figure 1.

The deviation of the double reciprocal plots *(1/v versus 1/S)* from linearity is difficult to interpret because the slope of the lines is a function of Km (slope = Km/Vm) and the curvatures may be the result of more than one enzyme

[4] Not published.

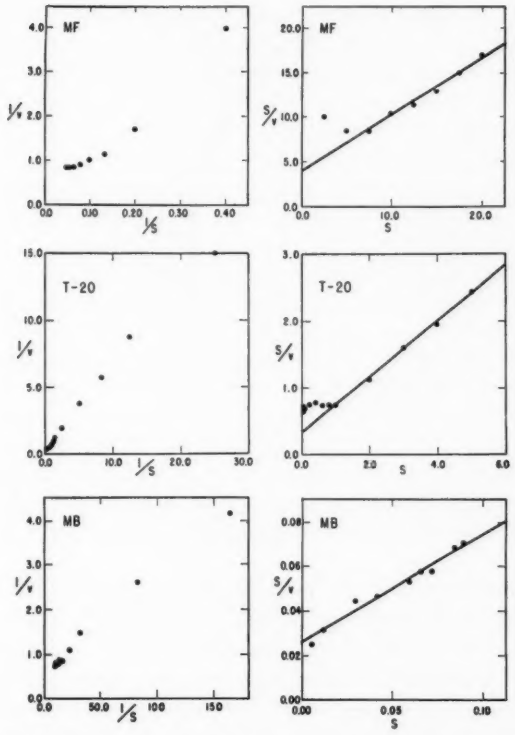

FIG. 1. Effect of substrate concentration *(S)* on the lipolytic activity *(v)* in milk toward milk fat (MF), Tween-20 (T-20), and methyl butyrate (MB).

Units, *S:* per cent for milk fat and Tween-20, moles per liter for methyl butyrate.

v: activity units per 100 ml. reaction mixture.

in the system. In the plot of S/v against S, however, the slope is exclusively a function of Vm (slope $= 1/Vm$) and any curvature can not be attributed to this factor. Hence, for the hydrolysis of milk fat and Tween-20 the abnormalities that occur at low substrate concentrations may be due to analytical errors involved at low activity determinations. Therefore, in the calculation of Km and Vm for these activities, more weight was given to the activity values obtained at high substrate concentrations. On the basis of the plots S/v against S, the Vm values for milk fat, Tween-20, and methyl butyrate activities were 1.6, 2.4, and 2.1 (units per 100 ml.), respectively, and the Km values were 6.3%, 0.81%, and 0.054 M, respectively. These constants of course have little meaning if more than one enzyme is involved in the reactions. In this case, they represent the resultant of all the enzymes concerned.

An attempt was made to estimate the possible heterogeneity of the enzyme system involved in the lipolytic activity in milk toward Tween-20 according

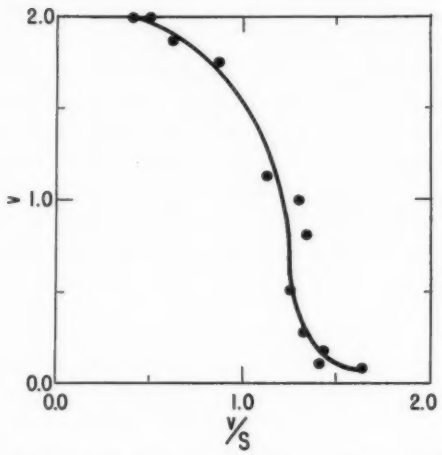

FIG. 2. A Hofstee plot for the lipolytic activity *(v)* in milk toward Tween-20 as influenced by the substrate concentration *(S)*.
Units, *S:* per cent Tween-20 in reaction mixture.
v: activity units per 100 ml. reaction mixture.

to Hofstee's treatment *(4, 5)*. In this case the complete solubility of this ester in water permitted activity determinations within a hundred-fold range of concentration. The plot of v against v/S (Figure 2) shows changes in the slope of the line with changes in the substrate concentration. Since the slope of this line is equal to the Michaelis-Menten constant (Km), the deviation in the linearity of this plot may be an indication, as proposed by Hofstee, that a multiple enzyme system is involved in this reaction. However, the shape of the curve obtained for Tween-20 is irregular in that the velocity of the reaction becomes abnormally slow at high substrate concentrations. This could be the result of micelle formation at high concentration of Tween-20, which is very surface active. Such an effect has been suggested by Hofstee *(6)* for the irregularity in the plot of v versus v/S obtained for the hydrolysis of *m*-heptanoyloxybenzoic acid by purified horse liver esterase. An alternative explanation for the abnormalities observed with Tween-20 is the possible adsorption of this ester, by virtue of its surface activity, around the active site(s) of the enzyme(s), thus causing steric hindrance.

It is evident that the value of kinetic data at variable substrate concentration to show the multiplicity of lipases in milk is limited. Abnormalities in the activity-substrate concentration curves may arise from surface effects. Additional complications are presented in heterogeneous systems, where a given change in substrate concentration may not necessarily result in the same change of effective surface area at the lipid-aqueous interface where hydrolysis occurs. Therefore the validity of this approach for milk fat hydrolysis *(13)* may be questioned.

The activation energy for the above activities was calculated by the Arrhenius equation in the range of 30 to 37° C., by using the data reported previously for

the relation of activity to temperature *(3)*. Values of 6,200, 14,500, and 12,500 calories were obtained for the lipolytic activity in milk toward milk fat, Tween-20, and methyl butyrate, respectively. These values are in line with those reported in the literature for lipases and simple esterases, respectively *(12, 14)*.

It has been suggested by Sizer *(14)* that when the activation energy for the action of an enzyme extract on two related substrates is the same, this may be taken as presumptive evidence that the same enzyme is catalyzing both reactions. However, in the case of esterases it is difficult to compare the activation energies obtained in homogeneous and heterogeneous systems, i.e., true solution and emulsion. Schönheyder and Volqvartz *(12)* found that the influence of temperature on the hydrolysis of *n*-propyl caprylate by pancreatic lipase in heterogeneous systems was negligible. They postulated that since the reaction in such systems occurs on a saturated surface, the rate would be little affected by concentration and temperature within wide limits. This would explain the relatively low value (about 4,500 calories) for the activation energy reported in the literature for lipase *(14)*.

To assess the specificity of the lipase system of milk, different mixed milks were surveyed for relative activity toward Tween-20, methyl butyrate, tributyrin, and milk fat. The data presented in Table 1 show a marked variation

TABLE 1

Relative lipolytic activity in different milks

Substrates[a]	Milks									
	1	2	3	4	5	6	7	8	9	10
	Activity *(units per 100 ml. milk)*									
T-20	3.91	4.37	3.41	3.95	4.22	3.75	3.02	3.16	2.65	4.48
MB	2.58	3.75	2.05	3.61	3.52	3.16	2.84	2.94	1.92	3.56
TB	11.1	14.2	8.77	19.0	20.5	13.4	14.2	11.7	12.1	18.7
MF	3.06	5.00	2.85	4.69	3.76	3.56	2.61	4.14	3.78	3.88
	Relative activity									
T-20	1.3	0.87	1.2	0.84	1.1	1.0	1.2	0.76	0.70	1.2
MB	0.84	0.75	0.72	0.77	0.94	0.89	1.1	0.71	0.51	0.91
TB	3.6	2.8	3.1	4.0	5.5	3.8	5.4	2.8	3.2	4.8
MF	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a] Abbreviations: T-20 = Tween-20; MB = methyl butyrate; TB = tributyrin; MF = milk fat.

in the relative lipolytic activity in ten different milks. The ranges in the activity values relative to milk fat were 0.60 for Tween-20, 0.43 for methyl butyrate, and 2.7 for tributyrin. The relative enzyme activity in a system is independent of the enzyme concentration. If one nonspecific enzyme is involved in milk, the relative lipolytic activity would be expected to remain constant in different milks. Therefore the variation in lipolytic activity observed in different milks may be considered as an indication of the multiplicity of lipolytic enzymes in milk. This evidence is only preliminary and will be substantiated on other grounds in the following papers of this series.

SUMMARY

The values obtained for maximum velocity (Vm) for the lipolytic activity in milk toward milk fat, Tween-20, and methyl butyrate were 1.6, 2.4, and 2.1 (units per 100 ml.), respectively, and the values for the Michaelis-Menten constant were 6.3%, 0.81%, and 0.54 M, respectively. The values for the activation energy in the range of 30 to 37° C. for the milk fat, Tween-20, and methyl butyrate hydrolyses were calculated to be 6,200, 14,500, and 12,500 calories, respectively. The kinetic data were considered in terms of a multiple lipase system in milk. The limitations of this approach were emphasized.

The variation in the relative lipolytic activity obtained in different milks towards different substrates is considered to be preliminary evidence for the presence of multiple lipolytic enzymes in milk.

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THE SPECIFICITY OF MILK LIPASE. III. DIFFERENTIAL INACTIVATION[1, 2]

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The relative lipolytic activity in a given milk toward different esters before and after various inactivation treatments (aging, heat, acid, alkali, trypsin, light, oxidizing agents, and formaldehyde) was studied. The results should lead to further work on the stability, separation, and purification of the enzyme system, which will aid in the problem of control of rancidity in milk and milk products. *Editor.*

In the previous paper of this series *(5)* preliminary evidence of the presence of multiple lipolytic enzymes in milk was obtained on the basis of variation in the relative lipolytic activity in different milks toward different esters. This paper presents further evidence of the multiplicity of lipolytic enzymes in milk.

Several workers have inferred the presence of more than one lipase in milk from variation in the inactivation of lipolysis in different milks by heat *(2, 19)*, formaldehyde *(6, 7, 8, 19)*, and copper *(6)*, and from variation in loss of tributyrinase activity from aging milk in the cold *(11)*. However, comparing different milks is difficult because of variations in environment and stability of milk lipase to aging in the cold *(17)*. Moreover, determining the extent of lipolysis and tributyrinase activity as measures of lipase concentration in milk may be questionable *(4)*. Some basic differences found in the conditions necessary to activate lipolysis in milk also have been attributed to the presence of multiple lipase systems *(16, 17, 18, 19)*.

The term lipase has been indiscriminately used by many authors to refer to the activity of milk toward tributyrin *(1, 10, 11, 12, 15)*. One would not seem justified to do so as long as no proof is available that the same enzyme or group of enzymes is measured by the milk fat and tributyrin hydrolyses. The multiplicity of lipolytic enzymes in milk would throw further doubts on the use of esters other than milk fat for lipase determinations in milk.

Because of the limitations of the kinetic approach for determining the multiplicity of esterases in a reaction system *(5)*, this problem was studied on

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[1] The data are part of a thesis presented to the Graduate School of the University of California by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, January, 1956.

[2] This study was supported in part by funds from the California Dairy Industry Advisory Board.

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another basis, that of differential inactivation of the lipase system of milk. This approach consists of studying the relative lipolytic activity in a given milk toward different esters before and after various inactivation treatments. It is based on the premise that if more than one lipase is present in milk, inactivation by different agents would be expected to alter the relative activity toward different substrates. Similar approaches have been used in systems other than milk *(3, 14)*. A wide variety of inhibiting agents were chosen. These treatments included: aging, heat, acid, alkali, trypsin, light, oxidizing agents, and formaldehyde.

MATERIALS AND METHODS

Freshly drawn mixed milk was used in all experiments. Because of the variation in stability of milk lipase upon aging in the cold *(17)*, all experiments were carried out with milk obtained within 1 or 2 hours after milking. Exposure of milk to light was soon found to cause considerable inactivation of milk lipase. Such exposure was therefore reduced to a minimum. The milk was skimmed by centrifugation and used as such.

The relative lipolytic activity in milk toward Tween-20 (T-20), methyl butyrate (MB), tributyrin (TB), and milk fat (MF) was followed by the methods developed in the first paper of this series *(4)*. All activity values are expressed in the present paper as enzyme units per 100 ml. of milk.

RESULTS

A number of preliminary experiments were carried out with each inactivation treatment under varying conditions. The treatments were chosen to decrease the hydrolysis of milk fat by about one half.

Effect of aging. Milk was aged under various conditions and the lipolytic activity toward different substrates was followed to assess the relative stability of the enzyme system involved. For this experiment, fresh milk was stored as skimmilk and as whole milk for different time intervals and temperatures. After storage the whole milk fractions were separated immediately before assaying for activity. Representative results from several stability experiments are given in Table 1. Storage of milk at 37° C. for 3 hours caused appreciable inactivation, the activity toward milk fat being more resistant than the other activities. After 48 hours of aging at 0-5° C., the per cent of loss in activity toward milk fat was again the least. It is significant to note that the lipase system of milk appears considerably more resistant to aging (24 hours) when stored as whole milk than as skimmilk.

The change in relative lipolytic activity that resulted from keeping whole milk at 37° C. for 3 hours and aging skimmilk at 0-5° C. for 24 hours provides preliminary evidence that different enzymes are involved in the activity in milk toward the different substrates. The enzyme system with greater affinity for milk fat appears more resistant to these treatments than that, or those, involved in the hydrolysis of the other substrates. The marked difference in specificity occurring

TABLE 1
Effect of aging on the relative lipolytic activity of milk

Storage conditions	Substrates[a]	Activity	Relative activity	Inactivation
		(*units per 100 ml. milk*)		(%)
Fresh milk	T-20	4.32	1.3	.....
	MB	2.50	0.75	.....
	TB	12.7	3.8	.....
	MF	3.32	1.0	.....
3 hrs. at 37° C.	T-20	2.52	1.1	42
	MB	1.49	0.64	41
	TB	6.59	2.8	48
	MF	2.32	1.0	30
3 hrs. at 0° C	T-20	3.91	1.3	9.5
	MB	2.58	0.84	0
	TB	11.1	3.6	13
	MF	3.06	1.0	7.8
24 hrs. at 0-5° C. as whole milk	T-20	3.77	1.5	13
	MB	2.12	0.85	15
	TB	11.1	4.5	13
	MF	2.50	1.0	25
24 hrs. at 0-5° C. as skimmilk	T-20	0.50	0.60	88
	MB	0.33	0.40	87
	TB	1.57	1.9	87
	MF	0.83	1.0	75
48 hrs. at 0-5° C. as whole milk	T-20	1.72	0.78	60
	MB	1.37	0.62	45
	TB	1.71	3.3	44
	MF	2.20	1.0	34

[a] Abbreviations used throughout the paper: T-20 = Tween-20; MB = methyl butyrate; TB = tributyrin; MF = milk fat.

in the whole milk and the skimmilk after aging for 24 hours indicates that different changes occur in the enzyme systems when milk is stored in the presence or absence of milk fat globules.

Effect of heat. Differential heat inactivation was studied by assaying for the different lipolytic activities before and after heating skimmilk at 50° C. for 15 minutes. For this purpose, 50 ml. of fresh skimmilk in 125-ml. Erlenmeyer flasks was brought rapidly to the desired temperature by constant shaking in a water bath adjusted to 55° C. At zero time the flasks were placed in a Warburg bath adjusted to 50.0° C. and the milk subjected to continuous but gentle agitation (to avoid stable foam formation) for 15 minutes. At the end of the heating period the samples were cooled within 30 seconds to 10° C. in a salt-ice mixture.

The results presented in Table 2 show the effect of this treatment on the relative lipolytic activity of three different mixed milks. The extent of inhibition of the lipolytic activity of milk toward the different substrates varied widely. In all cases the activity toward milk fat was the most resistant to the heat treatment. This can be attributed again to the contribution of different lipolytic enzymes in milk. The relative stability of these enzymes to heat corresponds to that observed with aging.

TABLE 2

Effect of heating (50° C. for 15 min.) on the relative lipolytic activity of milk

Enzyme sources	Substrates	Activity		Inactivation
		Control	Heated	
		(units per 100 ml. milk)		*(%)*
Milk 1	T-20	3.05	0.94	69
	MB	1.36	0.12	91
	TB	10.3	3.69	64
	MF	2.96	1.61	46
Milk 2	T-20	4.81	1.92	60
	MB	2.89	1.17	60
	TB	13.7	4.33	68
	MF	4.44	2.19	51
Milk 3	T-20	4.37	1.74	60
	MB	3.75	1.71	54
	TB	14.2	5.00	65
	MF	5.00	2.82	44

Effect of acid and alkali. For this experiment, appropriate amounts of 1 *N* NaOH or HCl were added to fresh skimmilk to obtain pH values of 6.0 and 8.9, respectively. A control was run at normal pH of milk containing an equivalent amount of water. No buffer was used and the final pH observed after treatment was taken as the experimental pH. The milks were incubated in Erlenmeyer flasks at 37° C. and shaken gently and continuously for 1 hour before assaying.

The results of a typical experiment are presented in Table 3. The loss in the activity toward milk fat resulting from these treatments was again the least. These results support the previous ones in indicating that different enzymes are present in milk that hydrolyze the above substrates. The enzyme system that hydrolyzes milk fat is more resistant than the enzyme(s) hydrolyzing the simpler esters. The fact that approximately the same extent of inactivation occurred at pH 6 as at pH 8.9 for each substrate suggests that the pH range of optimum stability for the enzymes involved was about the same.

Effect of trypsin. Two milks were incubated with 5 and 7 p.p.m. of crystalline trypsin,[4] respectively, at 37° C. for 1 hour with gentle agitation before assaying. The relative lipolytic activity toward the four substrates was determined before and after treatment.

The results (Table 4) reveal that the activities in milk toward tributyrin and milk fat were more sensitive to trypsin inactivation than the activities in milk toward Tween-20 and methyl butyrate. Although the activities toward Tween-20 and methyl butyrate were similarly affected, the difference between the activities toward tributyrin and milk fat may be significant.

Effect of light and oxidizing agents. Exposing layers of skimmilk to direct sunlight proved to be too severe. Therefore, for this experiment, two milks were exposed to diffuse daylight in 1-cm. layers in Petri dishes 14 cm. in diameter

[4] Obtained from Armour Laboratories, Chicago (1 mg. equivalent to 2500 "Armour Units").

TABLE 3
Effect of acid and alkali inactivation on the relative lipolytic activity of milk

Treatments	Substrates	Activity		Inactivation
		Control	Treated	
		(units per 100 ml. milk)		*(%)*
Acid: pH 6.0 for 1 hr. at 37° C.	T-20	3.95	1.71	57
	MB	3.61	1.16	68
	TB	19.0	6.36	67
	MF	4.69	2.47	47
Alkali: pH 8.9 for 1 hr. at 37° C.	T-20	3.95	1.61	59
	MB	3.61	1.10	70
	TB	19.0	6.24	67
	MF	4.69	2.80	40

for 30 and 60 minutes, respectively, at room temperature. In another experiment, two sets of samples of fresh skimmilk were treated with 2 p.p.m of hydrogen peroxide at room temperature and with 5 p.p.m. copper (as copper sulfate) at 37° C. for 15 and 30 minutes, respectively, before assaying.

The effect of these treatments on the relative lipolytic activity of milk is presented in Table 5. It is evident that the milk fat hydrolysis was less affected by oxidation than the other activities of milk. With photo-inactivation and treatment with hydrogen peroxide, approximately the same loss in activity occurred toward Tween-20, methyl butyrate and tributyrin in the milks studied. These results are in harmony with those obtained by aging and heat.

Effect of formaldehyde. Schwartz *et al. (13)* reported that formaldehyde acts as a competitive inhibitor toward milk lipase. To verify this finding, formaldehyde inhibition was studied in the presence or absence of the different substrates. For this purpose, small concentrations of formaldehyde were added in one case to the skimmilk and allowed to stand at room temperature for 1 hour before assaying and in the other case were added to the different reaction mixtures. The concentration of formaldehyde added to the reaction mixture was two times that used in the skimmilk alone. Since skimmilk constituted 40% of the

TABLE 4
Effect of trypsin inactivation on the relative lipolytic activity of milk

Enzyme sources	Trypsin Concentration[a]	Substrates	Activity		Inactivation
			Control	Treated	
	(p.p.m.)		*(units per 100 ml. milk)*		*(%)*
Milk 1	5	T-20	3.16	2.36	25
		MB	2.94	2.40	18
		TB	11.7	6.88	41
		MF	4.14	2.84	31
Milk 2	7	T-20	3.53	2.50	29
		MB	3.58	2.51	30
		TB	14.4	5.42	62
		MF	5.08	2.53	50

[a] Incubated at 37° C. for 1 hour.

TABLE 5

Effect of photo-inactivation and oxidizing agents on the relative lipolytic activity of milk

Enzyme sources	Treatment	Substrates	Activity		Inactivation
			Control	Treated	
			(units per 100 ml. milk)		(%)
Milk 1	Exposed 1 hr. to diffuse daylight	T-20	4.22	1.43	66
		MB	3.52	1.35	62
		TB	20.5	6.88	66
		MF	3.76	1.70	55
Milk 2	Exposed ½ hr. to diffuse daylight	T-20	3.75	1.20	68
		MB	3.16	1.20	63
		TB	13.4	4.80	64
		MF	3.56	1.90	47
Milk 2	H_2O_2: 2 p.p.m. for 15 min. at room temp.	T-20	3.75	1.20	68
		MB	3.16	1.07	66
		TB	13.4	4.32	68
		MF	3.56	1.62	55
Milk 3	Cu^{++}: 5 p.p.m. for 30 min. at 37° C.	T-20	3.89	1.98	49
		MB	3.48	1.66	53
		TB	16.3	9.60	41
		MF	3.44	2.20	35

reaction mixture, the concentration of formaldehyde per milliliter of skimmilk was five times that used in the skimmilk alone.

The effect of formaldehyde on the relative lipolytic activity of milk is shown in Table 6. Competitive inhibition is indicated with all four substrates, since the effect of formaldehyde was considerably diminished in the presence of these esters. The inhibition produced in the absence of substrates is of significance in showing differences in the sensitivity among the different lipolytic enzymes of milk.

Again the activity toward milk fat was more resistant than the activities toward other substrates when formaldehyde was added directly to skimmilk. The

TABLE 6

Effect of formaldehyde on the relative lipolytic activity of milk

Enzyme	Treatment	Substrates	Activity		Inactivation
			Control	Treated	
			(units per 100 ml. milk)		(%)
Milk 1	0.03% HCHO in skimmilk for 1 hr. at room temp.	T-20	4.48	0.88	80
		MB	3.56	0.70	80
		TB	18.7	3.66	80
		MF	3.88	1.42	63
Milk 2	0.06% HCHO in reaction mixt.	T-20	4.48	2.15	52
		MB	3.56	3.14	12
		TB	18.7	9.00	52
		MF	3.88	2.79	28
Milk 3	0.06% HCHO in reaction mixt.	T-20	4.22	1.88	56
		MB	3.52	2.64	25
		TB	20.5	7.52	63
		MF	3.76	1.52	59

relative resistance of these activities toward formaldehyde was similar to that toward other denaturing agents. This suggests that in addition to its competitive inhibiting effect, formaldehyde may also have a nonspecific denaturing effect on the enzyme proteins. Formaldehyde is known to combine with proteins with destructive effect. Further kinetic data should be obtained to determine definitely whether or not both competitive and noncompetitive inhibition are involved with formaldehyde. Such a study was beyond the scope of this investigation.

DISCUSSION

The evidence obtained in this study indicates that milk contains a multiplicity of esterases including both lipase and simple esterases. These enzymes were found to undergo different changes by inactivation. These results therefore throw serious doubts on the use of esters other than milk fat for lipase determinations. As long as these lipolytic enzymes in milk have not been separated, it is essential to use a substrate that will not confuse the lipase activity with its simple esterase activity.

Special attention was given in this study to the use of a large number of different inactivation treatments and their effect on the hydrolysis of widely different esters at optimum conditions for activity. The evidence was strengthened by the fact that the differential inactivation by such widely different treatments had similar effects on the relative lipolytic activities of milk. The activity in milk toward milk fat was consistently more resistant to all denaturing agents used than the activity toward simpler esters. Except for the relative effect of trypsin, these results seem to agree with those of Fodor for pancreatic lipase *(3)*. In his study the heat inactivation obtained was appreciably greater for the hydrolysis of simple esters than for the hydrolysis of simple triglycerides and olive oil.

Hoffman-Ostenhof *(9)* proposed to use the term "enzyme system" to indicate multiple enzymes. It is similarly suggested that the term "lipase system" be used to refer to the multiple lipolytic enzymes in milk. This term has already been used in the dairy literature without formal definition.

An interesting feature of the aging experiments was the marked increase in stability of the lipase system in the presence of fat globules when milk was aged in the cold as whole milk instead of as skimmilk. This observation suggests that some relationship may exist between milk lipase and the globular fat in whole milk that would protect the enzyme. The mechanism for this protection remains to be established and will be the subject of another study.

The knowledge obtained in this study on the specificity of milk lipase will permit further work on the stability, separation and purification of this enzyme system. Purification of the enzymes must be the ultimate aim in this field if detailed information on their physical chemical properties is to be obtained. A study of the specificity of lipase on the basis of partition among some milk components was carried out to substantiate the multiplicity of lipolytic enzymes in milk. This work will be presented in the next paper of this series.

SUMMARY AND CONCLUSIONS

The specificity of milk lipase was studied on the basis of the relative activity in milk toward different esters. Aging milk, heating it, and subjecting it to acid and alkali treatments, light, hydrogen peroxide, copper, and formaldehyde caused a greater decrease in its activity toward Tween-20, methyl butyrate, and tributyrin than in its activity toward milk fat. With trypsin inactivation, the activities toward milk fat and tributyrin were more affected than were the activities toward Tween-20 and methyl butyrate.

This differential inactivation is attributed to the presence in milk of multiple lipolytic enzymes of varying sensitivity. As long as these enzymes have not been separated, one would not be justified to use esters other than milk fat for lipase determinations in milk. The indiscriminate use of the term lipase to refer to the tributyrinase or simple esterase activity of milk is also not warranted. The adoption of the term "lipase system" is suggested to refer to the multiple lipolytic enzymes of milk.

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IDENTIFICATION OF CONSTITUENT AMINO ACIDS IN A PEPTIDE STIMULATORY FOR LACTIC ACID BACTERIA[1]

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A study was made to gain further information on the properties and composition of the peptides present in pancreas glands which stimulate the growth of lactic acid types of bacteria. The relationship between these active stimulants and strepogenin is discussed. *Editor.*

Certain peptides or crude extracts containing peptides have a stimulatory effect on the growth of various species of bacteria in the family *Lactobacteriaceae*. Interest in this general subject was intensified by the work of Woolley and his associates *(14)*, who coined the term "strepogenin" to describe these stimulatory peptides. The actual composition of strepogenin is unknown, although it has been presumed to arise as a result of hydrolysis of various proteins, and certain amino acids appear to be required for its activity *(15, 21, 22)*. Scott *et al. (13)* noted that the trypsin used to digest the protein also had strepogenin activity. Hankin *(3)* and Kizer *et al. (5)* observed that crude trypsin preparations contained two peptide stimulants whereas crystalline trypsin had no stimulatory effect. The stimulants present in crude trypsin appeared to be contaminants arising from the source of the enzyme preparations since the stimulants were also present in aqueous extracts of pancreas tissue.

The present study was undertaken to gain further information on the properties and composition of the stimulants present in pancreas glands. Such information is needed for a better understanding of the actual composition of strepogenin and the role of various peptides in the growth of lactic acid types of bacteria.

METHODS

Collection of pancreas glands and preparation of aqueous extract. Pancreas glands were removed from freshly slaughtered hogs, wrapped in heavy aluminum foil and immediately placed in contact with dry ice for freezing. The frozen glands were stored in a freezing cabinet and removed as needed for preparation of the aqueous extract. The frozen glands were placed in a meat grinder and thoroughly macerated. Distilled water was added at the rate of twice the pan-

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creas weight and mixed with the pulverized tissue by means of a Waring blendor; the resulting mixture was adjusted to pH 4.8-5.0 with 6 *N* HCl and heated in flowing steam for 20-30 minutes; the resulting precipitate was filtered from the cooled extract with the aid of Hyflo Supercel. The clear solution was then adjusted to pH 7.0 with 20 *N* KOH and heated to 112° C. for 6 minutes. This heat treatment caused precipitation of more solids, which were removed by filtration as before. The clear yellow solution, containing approximately 12-18 mg. total solids per milliliter was concentrated under vacuum to a solids content of 100-150 mg. per milliliter and placed in a freezing cabinet. After three successive periods of freezing and thawing, considerable amounts of denatured proteins were precipitated and removed by filtration. The clear filtrate was diluted with distilled water to a concentration of 40 mg. per milliliter. This solution will be referred to as "pancreas extract" in the remainder of the paper.

Assay of pancreas extracts in semi-synthetic medium. Hankin's modifications *(3)* of Kodicek and Mistry's medium *(6)* were adopted. The enzymatically hydrolyzed casein was prepared as described by Roberts and Snell *(12)*, except that 60 mg. of 2 × crystalline trypsin was used instead of pancreatin. The composition of the medium is presented in Table 1.

TABLE 1

Composition of semi-synthetic (H-KM) medium used for the microbiological assay of activity in pancreas extract

Component		Amount per 10 ml. final volume of medium
Acid hydrolyzed casein (in 10% solu.)		50 mg.
2X crystalline trypsin casein hydrolyzate		80 mg.
glucose		300 mg.
potassium acetate		20 mg. (to give a total of 100 mg.)
adenine, guanine, uracil and xanthine		0.1 mg. of each
thiamin hydrochloride		10 γ
riboflavin		10 γ
p-aminobenzoic acid		2.0 γ
niacin		10 γ
pyridoxin hydrochloride		25 γ
biotin		0.04 γ
calcium pantothenate		10 γ
folic acid		0.1 γ
K_2HPO_4 and KH_2PO_4		25 γ each
Salts E		0.05 ml.
	$MgSO_4 \cdot 7H_2O$ 60 mg.	
	$MnSO_4 \cdot 4H_2O$ 1.5 g.	
	NaCl 0.5 g	
	$FeSO_4 \cdot 7H_2O$ 4.0 mg.	
	$CuSO_4 \cdot 5H_2O$ 4.0 mg.	
	$ZnSO_4 \cdot 7H_2O$ 4.0 mg.	
	Dissolve in 250 ml. water and then add three drops of concentrated hydrochloric acid.	

The medium was prepared in double strength and dispensed in 5-ml. amounts into 18 × 150 mm. Pyrex culture tubes, covered with aluminum caps, and autoclaved at 112° C. for 6 minutes. After cooling the medium to room temperature, fractions for assay were added.

L. casei (A.T.C.C. ♯7469) was used as the test organism for all assays in the semi-synthetic medium. Stock cultures of this organism were carried in agar stabs of the semi-synthetic medium, and fresh stab cultures were prepared every 4 weeks. Cells of *L. casei* were grown for each assay by transferring one loop of cells from the agar stab into 10 ml. single strength broth for incubation at 35° C. for 24 hours. The cells from 4 ml. of this culture were harvested by centrifugation, washed with sterile saline (0.85%), and recentrifuged. The washed cells were then diluted with the saline so that a suspension giving 85% light transmission resulted. Per cent light transmission was read with a Lumetron Colorimeter (model 401) and a 580 mμ filter.

For the standard response curve, 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml. amounts of a 1:10 dilution of the pancreas extract were added. The volume was adjusted to 10 ml. with sterile distilled water and each tube was inoculated with one drop of the saline cell suspension delivered from a 1.0-ml. pipette graduated to the nearest 0.01 ml. Each assay tube was prepared in triplicate, one serving as an uninoculated control and the other two serving as duplicates of the particular level of diluted extract to be assayed. The tubes were thoroughly agitated and then incubated at 35° C. for 14 hours. Growth was measured turbidimetrically with the Lumetron Colorimeter and a 580 mμ filter.

Paper chromatographic techniques. Paper strips of Whatman ♯1 chromatography paper (3.8 cm. wide) were cut into 57-cm. lengths, and the desired amounts of pancreas extract (2.40 mg. or less) were applied to a base line located 10 cm. from one end. After the applied extract had dried, the strips were placed in a chromatography jar (30.5 cm. diameter by 61 cm. high) equipped with paper support rack and solvent assembly. The jar was then placed in an insulated cabinet and equilibrated for at least 6 hours, and the chromatogram was developed by descending solvent flow at room temperature (27°-29° C.) until the solvent reached a point 3 to 7 cm. from the end of the strip. All the chromatograms were dried in a forced draft at room temperature for at least 6 hours.

A 0.25% (w/v) solution of ninhydrin in anhydrous acetone as described by Toennies and Kolb *(17)* was used to detect the presence and resolution of alpha-amino type compounds in the pancreas extract. After development, a representative chromatogram was cut in half lengthwise. Half of the strip was dipped in the ninhydrin solution and then placed in an electric drying oven at 65°-70° C. until the color-reacting areas were clearly discernible. The remaining portion of the strip was examined under ultraviolet light and then bioautographed.

An ultraviolet lamp of 254 mμ wave length (model MC500-120-244, Research Equipment Corporation, Oakland, Calif.) was used to detect ultraviolet absorptive and fluorescent components present in the chromatogram of the pancreas extract.

Bioautographic techniques. A bioautographic technique described by Kennedy *et al. (4)* was used to detect areas of stimulation on the chromatograms. Bioautographs were prepared by using three semi-synthetic media. The modified medium of Kodicek and Mistry *(6)* described above was used, as well as

Niven's *(9)* semi-synthetic medium for *S. lactis* as modified by Collins *et al. (1)*. Also employed was the medium of Rabinowitz *et al. (11)*, which was modified in two respects: *(a)* the tryptic digest of casein was omitted and replaced by an equivalent weight of acid hydrolyzed casein, and *(b)* adenine, guanine, uracil, and xanthine were added at the rate of 0.1 mg. per milliliter single strength medium. In preparing the bioautograph plates, 125 ml. double strength medium was dispensed into a 500-ml. Erlenmeyer flask and heated in flowing steam for 30 minutes. The medium was tempered to 45°-50° C. and combined with 125 ml. of sterile 4.0% washed agar also tempered to 45°-50° C. (It was found that washing the commercial granulated agar eight to ten times with distilled water was necessary in order to insure a consistently clear medium for preparing the bioautograph plate.) Five ml. of sterile 0.05% brom cresol purple was then added to the mixture. Finally, 2.0 ml. of a 24-hour culture of *L. casei*, diluted with sterile 0.85% saline to 60% light transmission, was added. (When Niven's medium was used to prepare the bioautograph plates, 2.0 ml. of a 24-hour culture of *S. lactis*, diluted to 75% light transmission in the same manner, was added.) The flask was swirled to insure uniform mixing and the contents were poured into a 23 × 38 cm. Pyrex utility dish fitted with a glass cover. Once the medium had hardened, the strips to be assayed (cut lengthwise in half) were placed on the surface, where they remained for 10 minutes. The strips were then removed and the plates were placed in an incubator at 35° C. for 12-14 hours. *S. lactis* plates were incubated at 32° C. for 3.5-7 hours. The plates were observed periodically for the appearance of yellow zones due to increased acid production, which indicated the presence of stimulatory components.

The areas of the chromatograms that contained the stimulatory components were then cut out of the paper strip and eluted with methanol by refluxing for 1 hour.

The resolved active eluates obtained from chromatograms were hydrolyzed in 6 *N* hydrochloric acid and in saturated barium hydroxide in sealed Pyrex tubes at 121° C. for 15 hours. The acid hydrolyzate was evaporated to dryness on a steam bath three successive times to remove excess hydrochloric acid. The final volume was made to 1 ml. with water. In the alkaline hydrolyzates the excess barium was removed by the addition of a slight excess of sulfuric acid. The resulting precipitate was removed by centrifugation, and the solution was evaporated to dryness and reconstituted as described for the acid hydrolyzates.

Amino acids present in the acid and alkaline hydrolyzates were identified by using one- and two-dimensional paper chromatography. Known amino acids were prepared in a concentration of 5 mg. per milliliter in distilled water. Tyrosine and tryptophan were dissolved in 1 *N* NaOH and cystine in 2 *N* HCl. Approximately 0.005 ml. of each known amino acid solution was applied at 3-cm. intervals along the 10-cm. base line of an 18 × 57 cm. sheet of Whatman #1 chromatography paper. The hydrolyzates (0.005 ml.) were applied between different known amino acids. This allowed close comparison of Rf values and also of color reactions of the amino acids with ninhydrin after development in different sol-

vents. It was necessary to use more than one developing solvent to resolve fully all the amino acids in the hydrolyzates. Specific tests were made for certain amino acids. The hydrolyzates were tested for cystine and cysteine by the method of Toennies and Kolb *(17)* and for tryptophan by the method of Tauber *(16)*. The method of Feigl *(2)* was used to test for methionine on chromatograms of the acid hydrolyzates.

Once the identity of the amino acids present in the active eluate had been reasonably established, two-dimensional chromatograms of the acid hydrolyzates and known amino acids were prepared. These were developed with different solvents in a Chromatocab (Reco A300 Research Equipment Corporation, Oakland, California).

RESULTS

Microbiological activity of pancreas extract. The observation of Hankin *(3)* that an aqueous extract of hog pancreas glands was stimulatory for *L. casei* in the modified medium of Kodicek and Mistry *(6)* was confirmed. Figure 1 presents a typical response curve showing the stimulation afforded by the extract.

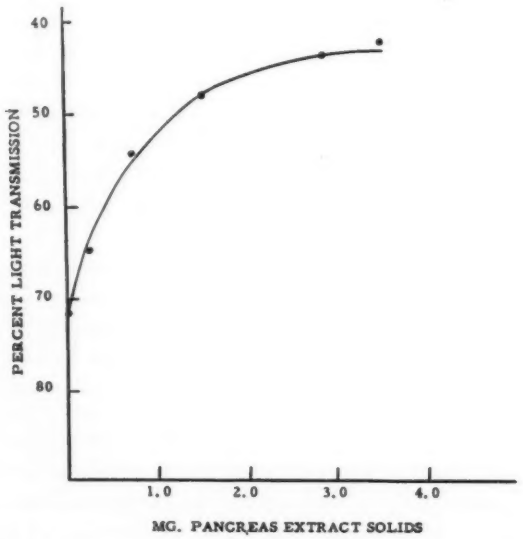

FIG. 1. Response of *L. casei* to pancreas extract in H-KM medium

Chromatographic and bioautographic techniques. Solvents used for the chromatographic separation of peptides and amino acids were employed in an effort to resolve stimulatory fractions present in the pancreas extract. Of a number tried, it was found that butanol, acetic acid, and water (40:10:50) described by Levy and Chung *(7)* provided resolution of an active factor at Rf 0.32. Another

active factor remained on the base line (Rf 0.05), and attempts to resolve it from other inactive components have been unsuccessful to date. The present investigation is concerned with the factor appearing at Rf 0.32. In the Kodicek and Mistry medium containing strepogenin (2 × crystalline trypsin hydrolyzate of casein) it was necessary to spot 9.60 mg. of pancreas extract solids on the paper chromatogram to detect the active factor, but resolution of the active factor from other ninhydrin-reactive compounds was poor. By spotting 2.40 mg. of pancreas extract solids on paper strips, resolution of the active stimulant from inactive components was markedly improved. The active spot on the latter chromatograms was readily detected on bioautographs made with the modified Rabinowitz medium to which no strepogenin was added. Therefore, in order to obtain resolution of the stimulant from inactive components, 2.40 mg. of pancreas extract was spotted on all further paper chromatograms and its location was detected on bioautographs made with the Rabinowitz medium. The biologically active area at Rf 0.32 gave a less pronounced color with ninhydrin than did many of the inactive bands. It was, however, fluorescent under ultraviolet light (254 mμ), and this property was used as a guide in removing the active area from the chromatogram for further study.

In bioautographs with *S. lactis*, active components were also observed at Rf 0.05 and 0.32 on chromatograms developed in butanol, acetic acid, and water (40:10:50).

TABLE 2

Rf values on one-dimensional chromatograms of known amino acids and ninhydrin reactive areas in hydrolyzate of active peptide

Butanol: acetic acid: water (40:10:50)			Pyridine: water (80:20)			Pyridine: acetic acid: water (50:35:15)		
Amino acid	Rf of amino acid	Rf of hydrolyzate spots	Amino acid	Rf of amino acid	Rf of hydrolyzate spots	Amino acid	Rf of amino acid	Rf of hydrolyzate spots
cystine[a]	0.06		lysine	0.20	0.19			
lysine	0.11	0.12	arginine	0.26		histidine	0.25	
histidine	0.14		aspartic acid	0.36	0.37	aspartic acid	0.26	0.26
arginine	0.16		glycine	0.38	0.39	glycine	0.28	0.28
serine	0.17	0.14	histidine	0.41				
aspartic acid	0.17		glutamic acid	0.42	0.42	glutamic acid	0.38	0.38
glycine	0.19	0.19	serine	0.49	0.49	threonine	0.44	0.44
hydroxyproline	0.20		alanine	0.52	0.53	alanine	0.45	
glutamic acid	0.22	0.23	threonine	0.53				
threonine	0.22		hydroxyproline	0.57				
alanine	0.24		valine	0.65	0.65	norleucine	0.62	
cysteine[a]	0.28					leucine	0.70	0.69
proline	0.29	0.27	norleucine	0.69	0.69	isoleucine	0.70	
tyrosine	0.36		isoleucine	0.69				
valine	0.42	0.45	leucine	0.69				
methionine[a]	0.45							
tryptophan[a]	0.51							
phenylalanine	0.54							
isoleucine	0.58	0.60						
leucine	0.58							
norleucine	0.62							

[a] Qualitative tests for these amino acids in the hydrolyzate were negative.

The active factor was cut from a number of chromatograms developed in the butanol, acetic acid, and water solvent and was eluted and concentrated; this eluate was then spotted on a second series of paper strips. Of various solvents tried, only 80% phenol (aqueous) separated the active area from nonactive, ninhydrin-reactive contaminants. In this solvent the factor appeared at Rf 0.95, was fluorescent, and was ninhydrin reactive. Therefore, acid and alkaline hydrolyzates were prepared of the eluate from a number of these resolved areas at Rf 0.95.

Hydrolyzates and known amino acids were chromatographed in various appropriate solvents (Table 2). Qualitative tests were made on the hydrolyzates for cysteine, cystine, tryptophan, and methionine and none of these amino acids was present; preliminary chromatographic analysis also indicated their absence in the hydrolyzates. By development in the butanol, acetic acid, and water solvent seven ninhydrin-reactive spots appeared, but only the spot for proline, which gave a characteristic yellow color, was resolved satisfactorily. The unresolved amino acids and hydrolyzate were then chromatographed in the pyridine, water solvent. These chromatograms showed the presence of lysine, serine, and valine and the absence of arginine and hydroxyproline; other amino acids were not resolved satisfactorily. Subsequently, chromatograms of hydrolyzate and unresolved amino acids were developed in pyridine, acetic acid, and water. These chromatograms showed characteristic Rf values for aspartic acid (which also gave a consistent characteristic blue color with ninhydrin), glycine, and glutamic acid; simultaneously no indication was found for the presence of histidine and norleucine. The remaining two ninhydrin spots indicated the presence of alanine and/or threonine and of isoleucine and/or leucine.

Two-dimensional chromatograms were then prepared of the hydrolyzed factor, the two unresolved pairs of amino acids, as well as the other amino acids found present by the previous one-dimensional chromatograms. Pyridine, acetic acid, and water (50:35:15) was used as the solvent mixture in the first dimension

TABLE 3

Rf values on two-dimensional chromatograms of known amino acids and ninhydrin reactive areas in hydrolyzate of active peptide

	First dimension pyridine: acetic acid: water (50:35:15)		Second dimension Butanol: acetic acid: water (40:10:50)	
Amino acid	Rf of amino acid	Rf of hydrolyzate spot	Rf of amino acid	Rf of hydrolyzate spot
lysine	0.29	0.30	0.10	0.10
aspartic acid	0.30	0.30	0.14	0.14
glycine	0.34	0.34	0.17	0.17
serine	0.38	0.39	0.17	0.18
glutamic acid	0.45	0.46	0.19	0.20
threonine	0.48	0.49	0.20	0.23
alanine	0.50	0.51	0.24	0.27
proline	0.53	0.52	0.31	0.32
valine	0.69	0.68	0.44	0.44
isoleucine	0.76	0.75	0.59	0.59
leucine	0.77	0.77	0.63	0.63

and butanol, acetic acid, and water (40:10:50) was used in the second dimension. By relative position and Rf values of the ninhydrin spots the following amino acids were found present: lysine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, valine, and probably both leucine and isoleucine (Table 3). The last two amino acids were not always completely resolved, but the chromatograms indicated the presence of two spots at the characteristic locations of leucine and isoleucine.

DISCUSSION

Although it is recognized that strepogenin is a peptide-like substance originating from tryptic hydrolyzates of a variety of proteins, the structure and arrangement of constituent amino acids in this substance are still unknown. Since it was discovered by Woolley *(19)*, considerable interest and significance have been attached to the role of strepogenin peptides in bacterial nutrition. Many proteins, after partial hydrolysis, have been categorized as to their relative strepogenin potency and their growth-promoting effects on various microorganisms *(6, 13, 14, 20)*. Some synthetic peptides have also been examined in this respect *(8, 10, 22)*.

Woolley *(21)* noted that strepogenin activity of insulin was due to the strepogenin structure being at the end of the peptide chain, and that glycine is the amino end-group of strepogenin. Later *(22)* working with synthetic peptides, he found that only those having a substituent amino acid on the amino group of glutamic acid were active. The substituent amino acids of these active peptides were serine, glycine, alanine, and tyrosine. Stokes *et al. (15)* provided evidence that indicated asparagine was closely related nutritionally to strepogenin; trypsin digest of casein inactivated by refluxing with HCl at pH 1.0 for 24 hours was completely inactive in an asparagine-free medium. The activity of 3.0 mg. of the strepogenin preparation, however, was fully restored when 0.75 to 1.0 mg. of L-asparagine was added per 10 ml. of medium. Woolley and Merrifield *(23)* have also reported the isolation of eight peptides, each showing high strepogenin activity for *L. casei*, from partial acid hydrolyzates of insulin. The most active peptide, which was 780 times as potent as the standard liver fraction "L," contained cystine, glutamic acid, glycine, serine, valine, and the leucines. The other peptides each contained the same amino acids with certain modifications; some contained tyrosine and some lacked one or two of glycine, serine, and isoleucine. It should be noted that the active peptide isolated in the present study contained all these amino acids with the exception of tyrosine and cystine, but in addition contained proline and threonine.

Scott *et al. (13)* observed that trypsin itself had strepogenin activity for *L. casei*. Later the studies by Kizer *et al. (5)* showed that crude trypsin preparations were stimulatory for *L. casei* in the presence of strepogenin and that these factors were present as contaminants in impure preparations of trypsin. Thus, it appeared that in many preparations of strepogenin, stimulants were derived from impurities in the enzyme used for making the hydrolyzate as well as from

hydrolytic products from the protein. Since the stimulants in crude trypsin have been shown by Hankin *(3)* to be those present in pancreas tissue, the peptide reported in the present study may have contributed to strepogenin activity in preparations made with crude trypsin. This may explain some of the differences reported in the characterization of strepogenin.

It is interesting to note that not only yeast extract but liver fraction "L," which has been used as a standard reference material in the quantitative assay of strepogenin, contained stimulants operative in the medium containing strepogenin (2 × crystalline trypsin digest of casein). On the basis of Rf values exhibited by these factors after chromatographic development in butanol, acetic acid, and water (40:10:50), it appeared that one may be the same as one of the stimulants of pancreas extract described here. This is being studied further.

It appears likely that with the eventual realization of the identity of strepogenin, no single structure will be found as the responsible entity of activity. Rather it seems that different peptides will be found stimulatory, depending on the actual amino acids present and their arrangement in the peptide molecule.

SUMMARY

Pancreas tissue was shown to contain two stimulants for *L. casei* and *S. lactis* which were active in the presence of strepogenin. One stimulant was purified chromatographically and showed characteristics of a peptide. Acid and alkaline hydrolyzates of the stimulant contained the following amino acids: lysine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, valine, and probably both leucine and isoleucine. The relationship between the active stimulants in pancreas tissue and strepogenin was discussed.

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THE RELATIVE USEFULNESS OF COMBINATIONS OF LABORATORY TESTS FOR PREDICTING THE FERTILITY OF BOVINE SEMEN

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It is important to know what combinations of laboratory tests are most useful for predicting the fertility of bovine semen and what percentage of the ejaculates must be discarded to achieve a specified increase in the nonreturn rate. The purpose of this study was to provide some preliminary quantitative estimates of these factors. *Editor.*

Numerous laboratory tests of semen quality have been reported to be correlated with fertility *(1, 2-7, 9-12, 14, 15, 17-21)*. In practice low spermatozoan concentration, low motility, long methylene blue and resazurin reduction times, and a high percentage of abnormal spermatozoa, have been used as bases for rejecting ejaculates which otherwise might have been considered satisfactory for breeding purposes.

However, the important consideration is not so much what correlation coefficients are statistically significant and at what level of probability but what combinations of laboratory tests are most useful for prediction purposes, and what percentage of the ejaculates must be discarded to achieve a specified increase in the nonreturn rate. To provide some preliminary quantitative estimates of these factors the investigations reported herein were conducted.

EXPERIMENTAL PROCEDURE

The following measurements were made on each of three ejaculates from each of 53 bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc.:

X_1. The concentration of spermatozoa in the unextended ejaculate immediately after collection as determined by the photoelectric method of Salisbury *et al. (15)*.

X_2. The per cent of motile spermatozoa in the unextended ejaculate immediately after collection as determined by the routine procedure of this laboratory *(22)*.

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X_3. The relative speed of forward movement of the spermatozoa in the ejaculate immediately after collection as determined by the routine procedure of this laboratory *(22)*.

X_4. The time in minutes required for 0.2 ml. of semen from the fresh ejaculate extended in 0.8 ml. of citrate-sulfanilamide-yolk *(16)* to reduce 0.1 ml. of a 50 mg.% solution of methylene blue in citrate-sulfanilamide buffer according to the method of Beck and Salisbury *(1)*.

X_5. The pH of the ejaculate immediately after collection.

X_6. The per cent of spermatozoa in the ejaculate immediately after collection that did not stain with eosin by the procedure of Lasley *et al. (10)*.

X_7. The per cent of morphologically abnormal spermatozoa in the ejaculate immediately after collection as determined from the stained smears used for X_6.

X_8 (1:100). The time in days required for the extended ejaculates to decrease to 30% motile spermatozoa when extended 1:100 in 3.6% citrate-sulfanilamide-yolk *(16)* and stored at 5° C. These values were determined from daily microscopic observations of subsamples taken from the extended ejaculates used for inseminating cows.

X_8 (1:300). The time in days required for the extended ejaculates to decrease to 30% motile spermatozoa when extended 1:300 in 3.6% citrate-sulfanilamide-yolk and stored at 5° C. These values were determined in the same manner as those for X_8 (1:100).

X_{9T}. The oxygen uptake in μl. per 10^8 spermatozoa per hour when extended at the rate of 1 part of semen to 4 parts of 3.6% citrate-sulfanilamide-yolk *(16)* and held at 37.5° C. for 1 hour. These measurements were made in conventional Warburg manometers within 3½ to 5½ hours after the ejaculate was collected. Until then the semen was held unextended at 5° C.

X_{9M}. The oxygen uptake in μl. per 10^8 motile spermatozoa per hour under the same conditions as for X_{9T}.

X_{10}. The milliliters of semen per ejaculate.

X_{11}. The millions of motile spermatozoa per milliliter of extended semen after extending the semen 1:100. These numbers were based on the values for X_1 and X_2, described above.

X_{12}. Millions of motile spermatozoa per milliliter of extended semen after extending the semen 1:300. These numbers also were based on X_1 and X_2.

Y_1. The per cent of first service cows not returning for reinsemination within 60 to 90 days after the month in which they were inseminated with semen extended 1:100 in 3.6% citrate-sulfanilamide-yolk.

Y_3. The per cent of first service cows not returning for reinsemination within 60 to 90 days after the month in which they were inseminated with semen extended 1:300 in 3.6% citrate-sulfanilamide-yolk.

The ejaculates used for breeding purposes were cooled slowly to 5° C. in 75 minutes prior to extending them with 5° C., 3.6% citrate-sulfanilamide-yolk *(16)*. They were then kept at 5° C.

Approximately one-half of each ejaculate was extended 1:100, and the remainder was extended 1:300. The inseminations were performed during the months of February, March, April, and May, 1948, and all technicians had equal opportunities to use the semen at the two extension rates.

RESULTS AND DISCUSSION

The means for each of the semen tests, the "gross" linear correlation coefficients for each test and fertility at the two extension rates, and the 60- to 90-day per cent nonreturns to a total of 12,953 first services for the two extension rates are shown in Table 1.

TABLE 1

Gross linear correlation coefficients between different semen tests and fertility

Semen quality test	Semen test code	Fresh ejac. means	1:100 dilution		1:300 dilution	
			Mean	"r"	Mean	"r"
10^6 sperm per ml. fresh ejaculates	X_1	1,310		+0.353**		+0.237**
% motile sperm in fresh ejaculates	X_2	65.5		+0.218**		+0.200*
Rate of sperm travel	X_3	3.2		+0.146		+0.157
Methylene blue reduction time (minutes)	X_4	8.0		−0.260**		−0.277**
pH of fresh semen	X_5	6.45		−0.181*		−0.216**
% unstained sperm in fresh semen	X_6	71		+0.198*		+0.187*
% abnormal sperm in fresh ejaculates	X_7	24.4		−0.241**		−0.199*
Livability in days at 5° C.	X_8		4.7	+0.150	3.2	+0.207*
μl. O_2 uptake per 10^8 sperm	X_{9T}	9.3		+0.267**		+0.169*
μl. O_2 uptake per 10^8 motile sperm	X_{9M}	14.1		+0.199*		+0.126
Volume of ejaculate	X_{10}	7.8		−0.170		−0.111
10^6 sperm per ml. extended semen	X_{11}		12.8	+0.352**	4.4	+0.253**
10^6 motile sperm per ml. extended semen	X_{12}		8.4	+0.386**	2.9	+0.290**
% 60- to 90-day NR to 7168 1st services	Y_1		56.6			
% 60- to 90-day NR to 5785 1st services	Y_2				52.8	

* Denotes statistical significance at the 5% level of probability.
** Denotes statistical significance at the 1% level of probability.

The difference of 3.8 percentage units between the average nonreturn rates at the 1:100 and 1:300 extension rates was highly significant statistically but actually is small in relation to the large difference in the average number of motile spermatozoa inseminated at the two extension rates. This difference in per cent nonreturns at the two extension rates is somewhat smaller than has been reported for similar extension rates by other investigators *(3, 4, 20, 21)*.

The "gross" rather than the "within" bull regressions were chosen for computing predicted nonreturns because the general procedure for discarding

ejaculates at the time of collection does not take into consideration the bull from which the ejaculate is obtained. If bulls themselves are to be culled because of low fertility, it would best be done on the basis of the nonreturns to a limited number of actual breedings, rather than on tests of semen quality. None of the correlation coefficients are large, although most of them are larger than required for significance at the 5% level of probability. The correlation coefficients between fertility and rate of spermatozoan travel, X_3, the livability of the spermatozoa at 5° C., X_8, their oxygen uptake, X_{9T} and X_{9M}, and the volume of the ejaculate, X_{10}, are either not significant statistically or are of low significance and too tedious to perform to be of practical value. Thus, there remain spermatozoan concentration, X_1, per cent motile spermatozoa, X_2, methylene blue reduction time, X_4, pH, X_5, per cent unstained spermatozoa, X_6, and per cent morphologically abnormal spermatozoa, X_7, as tests or combinations of tests for predicting nonreturn percentages under practical operating conditions.

F tests of the hypothesis that the regressions with respect to certain tests or combinations of tests = 0 revealed that only spermatozoan concentration, X_1, and per cent morphologically abnormal spermatozoa, X_7, contributed significantly to the prediction of fertility. The regression equation for the combination of these two tests is $y_o = 0.0144x_1 - 0.2618x_7$ for the 1:100 extension rate and $y_o = 0.0099x_1 - 0.2352x_7$ for the 1:300 extension rate, where the y_o's and x's are deviations about their respective means.

Since the percentage of morphologically abnormal spermatozoa, X_7, cannot be determined rapidly, the usefulness of this test at the time ejaculates are collected seems rather impractical. Furthermore, recent studies in this laboratory *(13)* indicate that the average percentage of abnormal spermatozoa in ejaculates collected now is slightly less than one-half that for the studies reported here and is not correlated with fertility. Thus it appears that spermatozoan concentration, X_1, is the most useful regression for predicting fertility; the equations, based on the data presented here, being $y_o = 0.0154x_1$ for the 1:100 extension rate and $y_o = 0.0108x_1$ for the 1:300 extension rate. Since the estimation of the per cent of motile spermatozoa may be done quickly, and certainly should be done routinely as a precaution against the use of ejaculates containing few motile spermatozoa, it is a simple task to combine spermatozoan concentration, per cent motile spermatozoa, and extension rate to give either the number of total or the number of motile spermatozoa per milliliter of extended semen, X_{11} and X_{12}, as a practical basis for predicting fertility.

If it is assumed that we are dealing with normal distributions with respect to the x's and the errors and that rejection of ejaculates is to be done strictly on the basis of their predicted fertility, a formula can be set up for computing the predicted increases in nonreturn percentages *(8)*. This formula is as follows: predicted increase = cD, where c is the estimate of the covariance between the predicted and the actual nonreturn percentages and D is a constant related to the fraction of ejaculates to be rejected. On the basis of the predicted increase in per cent nonreturns for different rejection rates and the mean nonreturn

TABLE 2

Examples of predicted average nonreturn percentages when different combinations of semen tests are used for rejecting ejaculates

Per cent of ejaculates to be rejected	Semen extended 1:100 and rejected on the basis of				Semen extended 1:300 and rejected on the basis of			
	X_1[a]	X_{12}[b]	X_1X_7[c]	$X_1X_2X_4X_5X_6X_7$[d]	X_1[e]	X_{12}[f]	X_1X_7[g]	$X_1X_2X_4X_5X_6X_7$[h]
0	56.6	56.6	56.6	56.6	52.8	52.8	52.8	52.8
10	57.7	57.8	57.9	58.0	53.6	53.9	53.8	54.0
20	58.6	58.8	58.9	59.1	54.2	54.8	54.5	55.0
30	59.4	59.7	59.9	60.2	54.8	55.6	55.3	55.9
40	60.3	60.6	60.8	61.2	55.4	56.4	56.0	56.8
50	61.2	61.6	61.9	62.3	56.0	57.3	56.8	57.7
60	62.1	62.6	63.0	63.6	56.7	58.2	57.6	58.7
70	63.2	63.8	64.2	65.0	57.5	59.3	58.6	59.9
80	64.6	65.3	65.8	66.7	58.4	60.6	59.8	61.4
90	66.7	67.6	68.2	69.3	59.9	62.6	61.6	63.6

[a] $y_e = 56.6 + 0.0154x_1$
[b] $y_e = 56.6 + 2.1645x_{12}$
[c] $y_e = 56.6 + 0.0144x_1 - 0.2618x_7$
[d] $y_e = 56.6 + 0.0172x_1 + 0.1650x_2 + 0.2595x_4 + 1.8269x_5 + 0.1763x_6 - 0.1902x_7$
[e] $y_e = 52.8 + 0.0108x_1$
[f] $y_e = 52.8 + 5.7431x_{12}$
[g] $y_e = 52.8 + 0.0099x_1 - 0.2352x_7$
[h] $y_e = 52.8 + 0.0029x_1 + 0.0780x_2 - 0.4462x_4 - 8.5840x_5 + 0.1185x_6 - 0.1834x_7$

percentages for the respective extension rates, predicted nonreturn percentages were computed for different combinations of semen tests and percentages of rejected ejaculates. These predicted percentages are shown in Table 2.

Two important relationships are to be seen in Table 2. One is the small differences among predicted nonreturn percentages for the combinations of semen tests shown, and the other is that rejection of 50% of the ejaculates on the basis of X_{12} and extending the remainder 1:300 results in a predicted nonreturn percentage essentially equal to that predicted when no ejaculates are rejected and all are extended 1:100. This latter relationship, if generally true, would have considerable importance because it would make possible a 50% increase in the number of potential breedings per ejaculate, without the small sacrifice in fertility normally expected from extending unselected ejaculates 1:300.

In order to determine which ejaculates should be rejected Table 3 has been constructed. It should be noted that in the upper range of values in Table 3, the nonreturn percentages below which ejaculates must be rejected to achieve a specified percentage of rejections is somewhat higher than the predicted average nonreturns of those to be retained for use. (See Table 2). This is a consequence of the fact that the prediction of fertility on the basis of semen tests is considerably less than perfect, and therefore the average of the ejaculates used tends toward the general mean rather than being beyond the rejection level.

For routine operations at a specified extension rate, such as 1:300, and with the rejection of ejaculates based on the number of motile spermatozoa per milliliter of extended semen, X_{12}, predicted nonreturn percentages for ejaculates of different quality may be computed and arranged as shown in Table 4.

TABLE 3

An example of predicted nonreturn percentages below which ejaculates must be rejected to achieve a specified percentage of rejections

Per cent of ejaculates to be rejected	When extending 1:100[a] reject ejaculates with predicted nonreturn percentages below	When extending 1:300[b] reject ejaculates with predicted nonreturn percentages below
10	35.9	31.0
20	43.0	38.5
30	48.1	43.9
40	52.5	48.5
50	56.6	52.8
60	60.7	57.1
70	65.1	61.7
80	70.2	67.1
90	77.3	74.6

[a] $m = 56.6; \sigma_a = 16.18$.
[b] $m = 52.8; \sigma_b = 16.99$.

If the relationship between fertility and the number of spermatozoa inseminated as found in these studies holds true at the higher nonreturn rates common today in artificial breeding—and there is some evidence to this effect *(2, 3, 4, 20, 21)*—it suggests that higher extension rates could be used provided they were accompanied by the use of effective tests for rejecting the less fertile ejaculates. As a consequence the usefulness of sires in artificial breeding could be greatly extended without sacrificing the present day high fertility levels. Further studies of this problem under present day operating conditions involving larger numbers of services per ejaculate and higher average fertility levels than obtained in these studies are to be reported later.

It should be recognized that the rejection of ejaculates may have undesired genetic consequences. For example, if ejaculates from sires with high production proofs in artificial breeding are culled more heavily than is compensated for by increased extension rates, the net result will be fewer progeny from these sires than might otherwise be possible. However, this situation can be

TABLE 4

An example of predicted nonreturn percentages (y_e) for ejaculates of different quality using the number of motile spermatozoa per milliliter of extended semen at 1:300 as the basis of rejection[a]

Concentration of spermatozoa in ejaculates $\times 10^6$/ml. (X_1)	% motile spermatozoa in ejaculates (X_2)				
	40	50	60	70	80
500	40.9	40.9	41.9	42.9	43.8
700	41.5	42.9	44.2	45.5	46.9
900	43.0	44.8	46.5	48.2	49.9
1100	44.6	46.7	48.8	50.9	53.0
1300	46.1	48.6	51.1	53.5	56.1
1500	47.6	50.5	53.4	56.2	59.1
1700	49.2	52.4	55.7	58.9	62.2
1900	50.7	54.4	58.0	61.6	65.3
2100	52.2	56.2	60.3	64.3	68.3

[a] $y_e = 52.8 + 5.7431x_{12}$ where x_{12} is the deviation from the mean value of 2.9×10^6 motile sperm per milliliter of extended semen at 1:300.

corrected by combining the appropriate genetic data with semen and fertility data to arrive at a prediction of maximum genetic gain.

If a large number of young sires are being sampled in a production testing program, it is quite likely that more semen will be available than is needed. Consequently, the young sires' ejaculates may be culled rather rigorously on the basis of predicted fertility with little likelihood of restricting the use of the young sires to the point where an early proof of genetic merit for production and other economic traits will be greatly delayed.

SUMMARY AND CONCLUSIONS

Statistically significant "gross" linear correlations were obtained between the fertility of bovine ejaculates extended 1:100 and 1:300 and the concentration of spermatozoa in the ejaculate, the per cent of motile spermatozoa, the methylene blue reduction time, the pH, the per cent of unstained spermatozoa, the per cent of morphologically abnormal spermatozoa, the livability of spermatozoa at 5° C., and the oxygen uptake of spermatozoa at 37.5° C. The average 60- to 90-day per cent nonreturns to 7,168 first services with semen extended 1:100 and 5,785 first services with semen extended 1:300 in 3.6% citrate-sulfanilamide-yolk were 56.6 and 52.8, respectively.

From among the semen tests that may be performed quickly after the ejaculate is collected, spermatozoan concentration and the per cent of abnormal spermatozoa make substantial contributions to the prediction of per cent nonreturns at extension rates of either 1:100 or 1:300. However, in the routine examination of semen for large scale artificial breeding operations, the culling of ejaculates on the basis of their predicted nonreturns from the number of motile spermatozoa per milliliter of extended semen appears to be more feasible.

Rejecting 50% of the ejaculates by this procedure and extending the remaining ones 1:300 resulted in a predicted average nonreturn percentage equal to that predicted when no ejaculates were rejected and all were extended 1:100. The practical consequence of the use of such a culling and extending procedure would be to make available for breeding purposes 50% more extended semen without sacrificing fertility. Results of further studies of this problem under present day operating conditions and fertility levels will be reported later.

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ESTIMATING THE HERITABILITY OF TYPE CLASSIFICATIONS OF DAIRY CATTLE

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There is a question as to whether or not the rating values as used in type classification should be equidistant. A sample of Jersey type classification data was used as a basis for this mathematical study of the problem, which involved 2,810 daughter-dam pairs. The results indicate that an arbitrary system of consecutive numerical scores was as accurate as scores derived from the data. *Editor.*

The rating of Jersey cattle for conformation (type) was inaugurated by The American Jersey Cattle Club in 1932. Under this plan of herd classification, an official inspector rates the cows according to the rules of the classification program. The cows are given a single over-all rating in one of six grades: Excellent, Very Good, Good Plus, Good, Fair, and Poor. By rule of The American Jersey Cattle Club, the certificate of registration is canceled if cows classify Poor; therefore, in studying the type data from the Breed Association, one is concerned with the five remaining classes.

In order to make a numerical analysis of type classification data, the usual procedure is to assign consecutive integers to the ratings as follows: Fair = 2, Good = 3, Good Plus = 4, Very Good = 5, and Excellent = 6. The question arises whether or not these scores should be equidistant apart. Fisher *(2)* has outlined a method for determining the appropriate numerical values to be assigned to non-numerical data. Rae *(5)* suggested that maximizing the regression

TABLE 1
Frequency distribution of the type classifications of dams and daughters included in these data and for the Jersey breed

Rating	Dams		Daughters		Breed percentage of total[a]
	(No.)	*(%)*	*(No.)*	*(%)*	
E	98	4.3	37	1.3	2.6
VG	992	44.5	962	34.2	30.5
GP	903	40.6	1,330	47.4	46.8
G	215	9.7	422	15.0	17.5
F	19	0.9	59	2.1	2.6
Total	2,227	100.0	2,810	100.0	100.0

[a] This is the distribution of the classifications of 170,827 Jersey females classified during the period 1943-1953.

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of daughter on dam would give the appropriate numerical values to be used in estimating the heritability of sheep scores. The present paper shows the results of applying these methods to a sample of Jersey type classification data. The problem of unequal numbers of daughters per dam encountered when estimating heritability by the regression of daughter on dam is also considered.

DATA

The type records for this analysis were provided by The American Jersey Cattle Club in conjunction with another study *(7)*. The data consisted of 2,810 daughter-dam pairs from 414 herds with the daughters being the progeny of 756 sires. The frequency distribution of the type classifications of the dams and daughters as well as that for the Jersey breed are shown in Table 1. There is good agreement between the breed distribution and the distribution for the daughters, indicating that the daughters were a representative sample of the breed. The dams had somewhat higher frequencies in the Excellent and Very Good ratings and correspondingly lower frequencies in the Good Plus and Good ratings. This is to be expected because of a greater opportunity for selection of dams and since the ratings can be raised but not lowered on subsequent reclassification.

EXPERIMENTAL PROCEDURE AND RESULTS

Estimating type scores. The mathematical model

$$y_{ijk} = \bar{y} + b\,(x_{ij} - \bar{x}_{i.}) + e_{ijk}$$

was used to express the intra-sire regression of daughter on dam. In the model, y_{ijk} is the type rating of the k^{th} daughter of the j^{th} dam and the i^{th} sire, x_{ij} is the type rating of the j^{th} dam mated to the i^{th} sire and b is the intra-sire regression of y_{ijk} on x_{ij}. The intra-sire regression, b, is the ratio of the within sire crossproducts, P, and the within sire sums of squares for dams, S. The values for P and S are expressed in terms of t_i where the five type classifications of Excellent, Very Good, Good Plus, Good, and Fair are denoted by t_5, t_4, t_3, t_2, and t_1, respectively.

The intra-sire regression, $b = P/S$, can be expressed in terms of the t_i's as follows:

$$\frac{P}{S} = \frac{\sum\limits_{ij} n_{ij.}\, t_i\, t_j - \sum\limits_{k} \dfrac{\sum\limits_{i=j}\sum n_{ik}\, n_{jk}\, t^2{}_i + \sum\limits_{i\neq j}\sum n_{ik}\, n_{jk}\, t_i\, t_j}{n_{.k}}}{\sum\limits_{i} n_{i.}\, t^2{}_i - \sum\limits_{k} \dfrac{\sum\limits_{i} n^2{}_{ik}\, t^2{}_i + \sum\limits_{i\neq j}\sum n_{ik}\, n_{jk}\, t_i\, t_j}{n_{.k}}} \qquad (1)$$

where the i and j subscripts refer to the different t classes and the k subscript refers to sires. The analysis consists of obtaining values for the t_i's that will maximize b. The partial derivative of $b = P/S$ taken with respect to each of the t_i's can be reduced to form the expression

$$\left[\frac{\partial P}{\partial t_i} - b\,\frac{\partial S}{\partial t_i}\right] \qquad (2)$$

The $\frac{\partial P}{\partial t_i}$ and $\frac{\partial S}{\partial t_i}$ are systems of quadratic equations. Since they are not independent, in order to get a solution, it is necessary to impose the restriction that $t_1 = 0$. The largest value of b which will make equation 2 equal to zero is the solution desired and it can be found by an iterative method developed by Aitken *(1)* and used by Rae *(5)*. Applying this procedure to the data resulted in a value of $b = 0.14$. In order to solve for the values of t_2, t_3, and t_4, the value of t_5 was set equal to 1.0. The solution for the other t_i's was: $t_2 = 0.19$, $t_3 = 0.51$, and $t_4 = 0.71$. The corresponding values obtained by making the analysis on a within herd basis (instead of intra-sire) were $t_2 = 0.08$, $t_3 = 0.51$, and $t_4 = 0.78$ with a maximized b value of 0.15.

The values of the t_i's were tested to see if they differed significantly from an arbitrary scoring system where $t_1 = 0$, $t_2 = 0.25$, $t_3 = 0.50$, $t_4 = 0.75$, and $t_5 = 1.0$. Analysis of covariance to adjust the set of scores derived from the intra-sire analysis and then an analysis of variance of the adjusted scores *(2)* as given in Table 2 indicated that there was a nonsignificant difference between the empirical and arbitrary scores. The same was true for a similar analysis of the intra-herd t values. The intra-sire correlation between the arbitrary and the adjusted t values was 0.99 and is highly significant. Thus, the procedure of coding type ratings such that Fair = 2, Good = 3, Good Plus = 4, Very Good = 5, and Excellent = 6 seems valid for estimating the heritability of type scores in dairy cattle.

TABLE 2

Analysis of covariance for the arbitrary scores (Y) and the empirical scores (X) and the test of significance of the adjusted empirical scores

Source of variation	S(Y²)	S(YX)	S(X²)	Degrees of freedom	Adjusted sums of squares	Mean square
Sires	14.949	14.862	14.847	758	0.0764	0.0001
Remainder	95.663	93.132	93.042	2,051	2.3745	0.0012
Total	110.612	107.994	107.889	2,809	2.4509	

The intra-sire regression of daughter on dam should contain about one-half of the additive genetic variance plus a small fraction of the epistatic variance. Therefore, the derived t values do not maximize the ratio of the additive genetic variance to the total variance unless the epistatic variance is zero. Doubling the regression coefficient, $b = 0.14$, gives an intra-sire heritability of type of 0.28. The intra-sire estimate obtained from an analysis using the arbitrary scores was 0.25. This difference is not significant and is largely the result of the empirical scores being a better fit to the model.

Variable numbers of daughters. There were 463 dams in these data that had from two to six offspring. The number of dams having 2, 3, 4, 5, and 6 daughters was 362, 86, 12, 2, and 1, respectively. These daughter groups included sets of half sibs, full sibs, and some of both. The proper weighting of dams with variable numbers of offspring in computing the regression of progeny mean on parent has

been calculated by Kempthorne and Tandon *(3)*. This method has been more clearly stated by Reeve *(6)* and expanded to include families of full sibs as well as mixed families. The principle of choosing the weights such that the regression has minimum sampling variance was followed. By using the method suggested by Kempthorne and Tandon, and Reeve, the intra-herd regression of daughter on dam was found to be 0.145.

Legates and Lush *(4)* used the expectation of the number of $\sigma^2 G/2$ terms in the crossproduct between each daughter's record and the dam's record to adjust the degrees of freedom for those dams with more than one daughter. This gives rise to fractional degrees of freedom for the crossproducts terms of the covariance analysis. The expected numbers of degrees of freedom for such an analysis are

TABLE 3

The expected number of degrees of freedom in a covariance analysis of data including dams with variable numbers of daughters

Source	Expected degrees of freedom
Total	$n_{\ldots} - \dfrac{\sum_{ij} n^2_{ij.}}{n_{\ldots}}$
Among herds	$\sum_i \dfrac{\sum_j n^2_{ij.}}{n_{i..}} - \dfrac{\sum_{ij} n^2_{ij.}}{n_{\ldots}}$
Within herds	$n_{\ldots} - \sum_i \dfrac{\sum_j n^2_{ij.}}{n_{i..}}$

listed in Table 3 where $n_{\ldots}$ is the total number of daughters and $n_{ij.}$ is the number of daughters of the j^{th} dam in the i^{th} herd. The method of expectation or fractional degrees of freedom resulted in an intra-herd regression of daughter on dam of 0.148.

TABLE 4

Methods of estimating the intra-herd regression of daughter on dam and the corresponding heritability of type

Type of regression	Regression Coefficient	Intra-herd heritability
Repeating the dam's record with each daughter's record	0.145	0.291
Unweighted regression of means of daughters' on dams' record	0.146	0.293
Weighted regression	0.145	0.291
Expectation	0.148	0.296

Given in Table 4 are the intra-herd regressions of daughter on dam resulting from four methods of computation. Because of the similarity of the intra-sire and intra-herd estimates of the maximized b value of the previous section, the regressions in Table 4 were computed only on an intra-herd basis. It is evident that the differences among the four estimates are small. This is not surprising since nearly 80% of the data were from dams with only one daughter and the intra-herd correlation between records of daughters of the same dam was 0.07

for half sibs and 0.15 for full sibs. Unless one has a sample of data in which most of the cows have more than one daughter or there is a higher intra-class correlation between the type ratings of the daughters of the same dam than was found here, there is little to be gained in using the more complicated methods of estimating the heritability of type conformation.

The difference between the unweighted regression of means of daughters on dam's record and the weighted regression was less than that reported by Kempthorne and Tandon *(3)*, although the dams in their data averaged 1.39 daughters per dam as compared to 1.26 here. They found that the weighted regression gave about the same result as repeating the dam's record with each daughter's record, which is also consistent with the present findings. The ease with which the dam's record can be repeated with each daughter's record makes this method the most desirable from the standpoint of computation. On the other hand, for a large volume of data, one would probably be justified in choosing at random one daughter from those dams with more than one daughter. If the selection of the daughter is random, the regression coefficient should not be affected.

SUMMARY

An analysis of type classification data involving 2,810 daughter-dam pairs was made to determine what numerical values the five grades of Excellent, Very Good, Good Plus, Good, and Fair should be given when estimating the heritability of type. The results indicated that an arbitrary system of consecutive numerical scores was as accurate as scores derived from the data.

Estimates of heritability of type were obtained by including all of the daughters of a dam in the covariance analysis. The following four methods of calculating the regression coefficients were compared: 1 and 2, the weighted and unweighted regression of the means of daughters records on dam's record, 3, repeating the dam's record with each daughter's record, and 4, adjusting the degrees of freedom in the crossproduct terms for the dams with more than one daughter. The resulting heritability estimates were approximately the same; consequently, the method of repeating the dam's records with each daughter's record seems sufficiently accurate and has the advantage of being easy to compute.

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CAROTENE IN THE RATION OF DAIRY CATTLE. II. THE INFLUENCE OF SUBOPTIMAL LEVELS OF CAROTENE INTAKE UPON THE MICROSCOPIC ASPECT OF SELECTED ORGANS[1,2]

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The influence of suboptimal intakes of carotene on the structure of the pituitary, adrenal, thyroid, ovarian, and testicular tissues of dairy cattle and newborn calves has been studied with eight Jersey and four Holstein cows as the foundation animals. Postmortem examinations were made on all animals of the second and third generation group that died or were slaughtered. From the results it is concluded that recommended vitamin A and carotene allowances for dairy cattle may require modification. *Editor.*

It is well established that carotene and/or vitamin A deficiencies affect the structure of tissues and organs. The data have been obtained for the most part from small laboratory animals and to a lesser extent from bulls and newborn calves from cows fed carotene and/or vitamin A-deficient rations. Little information is available on the effect of suboptimal carotene and/or vitamin A intake upon the histological aspect of tissues, particularly when the deficient ration has been fed for extended periods of time during the lifespan of individuals, or through two or three generations.

Numerous workers *(2, 3, 4, 7)* have reported cystic pituitaries and degenerative changes in the testicles of bulls on carotene-deficient diets. Erb and coworkers *(2, 3)* demonstrated histopathological changes in the anterior pituitary and adrenals of bulls on a carotene-deficient ration. Jungherr and associates *(6)* reported consistent changes in the anterior pituitary and a decrease in the number of chromophilic cells. Marked hyperplasia of the parotid salivary glands and mild hyperplasia of the thyroids also were observed. Moore *(8, 9)*, employing an ophthalmoscope, described mottling of the tapetum lucidum or tapetum nigrum in the eyes of carotene-deficient animals. Papilledema of the nerve head of the optic nerve was frequently seen. In calves the optic nerve often showed degeneration at the point where it passed through the optic foramen *(5, 8)*. This was not observed in mature cows *(9)*. Thorp *(10)* reported that

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calves on extremely low carotene rations at times showed degenerative and inflammatory changes in the kidneys. With the exception of cystic pituitaries, however, histological changes in the female bovine induced by carotene deficiency have not been reported.

This paper reports the influence of suboptimal (e.g. limited, but not regarded as deficient) intakes of carotene upon the structure of the pituitary, adrenal, thyroid, ovarian and testicular tissues of dairy cattle and newborn calves from animals maintained for extended periods of time on low carotene rations.

EXPERIMENTAL PROCEDURE

A group of eight Jersey and four Holstein cows, all pregnant and raised on a normal ration, were selected as the foundation animals for this experiment. They were placed on a ration of grain and low carotene roughage for a period of three to eight months during the latter period of pregnancy. Two of the Jersey cows were fed a low-carotene ration for seven months of their second gestation.

The roughages varied somewhat throughout the experiment but contained only 1 to 4 p.p.m. of carotene. Carotene supplements in the form of alfalfa meal or carotene-rich oil were fed in such quantity as to ensure the animals a daily intake of 50 γ of carotene per kilogram of body weight. Sufficient grain, roughage, and mineral supplements were fed to supply the required amount of digestible protein, total digestible nutrients, and mineral elements.

Eight female and six male calves were born to the first generation cows and, after an initial 45-day milk feeding period, were placed on the same level of carotene as had been fed their dams. These calves were designated as the second generation low-carotene group, and the females were placed on the breeding list at 15 months of age. Added to this group was one heifer calf (H557) whose dam was not fed the low-carotene ration during pregnancy. The calves from the second generation low-carotene animals formed the third generation low-carotene group.

Post-mortem examinations were made on all animals of the second and third generation group that died or were slaughtered. Tissues were obtained as quickly after death as possible, fixed in Bouin's fluid, embedded in paraffin, sectioned at 10 μ, and stained with hematoxylin-eosin.

RESULTS

The first generation cows produced apparently normal calves. The only deviation observed was that the birth weights were somewhat lower than the average for their respective breeds.

The second generation group showed a marked loss of reproductive efficiency. Although brucellosis-free, these animals frequently aborted or produced stillborn calves or calves too weak to survive. During a 28-month calendar period when the second generation group was fed a ration supplying 50 γ carotene daily per kilogram of body weight, only two normal calves were produced. An

additional calf (G-13) was the offspring of H557, whose dam had not been a member of the first generation group but which had been carried on the low-carotene ration. Subsequently H557 produced a second normal calf (H557B1) while on the low-carotene ration.

After the 28-month period, the ration of the second generation group was raised to a level of 90 γ carotene daily per kilogram of body weight for a period of 13 months. During this period five calves were born, four of which were abnormal or dead at birth.

The carotene level was again raised to a daily intake of 130 γ per kilogram of body weight for a period of 18 months. During this period three calves were born. Two of these (H557B1 and J267B1) were normal, but the other (J251B3) was 6 to 8 weeks premature, weak and blind and died shortly after birth.

One animal (H553), upon being fed a normal ration for 10 months, produced a stillborn male calf (H553B2) which showed post-mortem lesions indicative of strangulation in the birth canal. A previous male calf (H553B1) born to this dam also was stillborn and showed similar lesions. Probably some physical or anatomic defect contributing to dystocia was the cause of these deaths, but this was not satisfactorily proven by physical examination.

TABLE 1

Macroscopic and microscopic observations of low carotene calves dead or abnormal at birth

Experimental animal	Carotene intake of dam during gestation		Age at death or slaughter	Condition of calf at birth
	(γ/kg.)	*(days)*	*(days)*	
Females				
G-5	90	260	8	Weak, blind, constricted and degenerated optic nerve, protruding Descomet's membrane, corneal opacity, abnormal front legs, slightly hydrocephalic.
G-8	90	267	6	Weak, blind, protruding Descomet's membrane, corneal opacity, constricted and degenerated optic nerve, hydrocephalic.
Males				
J253B1	50	277	3	Weak, blind, constricted and degenerated optic nerve, abnormal front legs, slightly hydrocephalic.
J255B1	50	204		Stillborn, slightly degenerated optic nerve.
H553B1	90	278		Stillborn, slightly degenerated optic nerve.
J250B1	50	233	1	Weak, abnormal, hydrocephalic, constricted and degenerated optic nerve.
J251B1	50	261		Stillborn, hydrocephalic, blind, constricted and degenerated optic nerve.
J251B2	90	278	1	Weak, abnormal, blind, constricted and degenerated optic nerve.
J251B3	130	210	2	Stillborn, constricted and degenerated optic nerve.

Macroscopic and microscopic observations of autopsied calves. Observations on calves stillborn or dying shortly after birth are presented in Table 1. Five calves showed evidence of hydrocephalus and either were born dead or were too weak to survive. Figure 1, showing the cross-section of the skull of calf G-8,

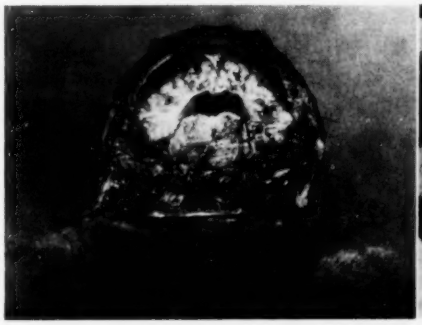

FIG. 1. Cross-section of skull of G-8 showing internal hydrocephalus.

FIG. 2. Head of G-8 showing corneal opacity.

demonstrates the typical mild internal hydrocephalus and distortion of the vault which were found. Blindness, as determined by ophthalmoscope examination after the method of Moore *(8)*, was frequent. A number of calves showed evidence of papilledema and mottling of the tapetum lucidum and tapetum nigrum. Steamy corneas, with subsequent opacity, erosion, and protrusion of Descomet's membrane, were seen in some animals (Figure 2). Constriction of

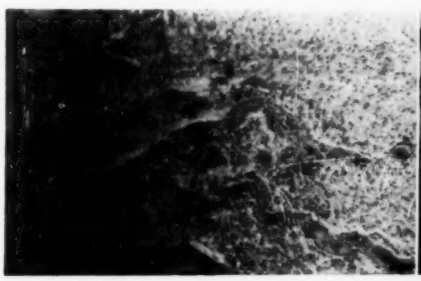

FIG. 3. Optic nerve showing necrosis.

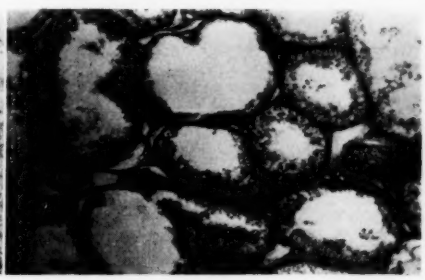

FIG. 4. Left testicle of Holstein bull 500B at slaughter.

the optic foramen, with resultant damage to the optic nerve, the cellular structure revealing the characteristic pyknosis and loss of structural integrity characteristic of pressure necrosis, was consistently observed (Figure 3).

Microscopic cysts were observed in the pituitaries of three third generation calves. Third generation Holstein bull calf H557B1, born to a dam receiving daily carotene intakes of 130 γ per kilogram of body weight and itself receiving

the same carotene level after the colostrum period until slaughtered at 4 months of age, showed multiple microscopic cysts of the pars nervosa. Third generation Jersey bull J267B1 was slaughtered at 10 months of age. His dam also received the 130 γ per kilogram carotene ration, but the calf received a daily carotene intake of 390 γ per kilogram of body weight after colostrum feeding until time of slaughter. Examination of the tissues revealed no apparent abnormalities except for the pituitary, which showed multiple microscopic cysts in the pars nervosa, and the testes, which were infantile for the age.

Examination of the thyroids, adrenals, ovaries, and testicles of autopsied calves failed to reveal any abnormal condition that could be attributed to the deficiency diets of the dams.

Histological findings in older animals. Jersey bull J218B and Holstein bull H496B were slaughtered at 16 to 18 months of age. These animals had been maintained on a ration of 50 γ per kilogram of body weight per day of carotene. The tissues were essentially normal with the exception of the testicles, which showed reduced spermatogenesis, and the pituitary of H496B, which showed microscopic cysts in the pars nervosa.

Jersey bull J221B and Holstein bull H500B were slaughtered at approximately 4 years of age. These animals were maintained on a ration of 50 γ of carotene per kilogram of body weight per day after the milk feeding period to almost 2½ years of age, at which time they were increased to 90 γ per kilogram for the period from 2½ to 3½ years of age. The carotene intake was increased further to 130 γ per kilogram for the 7 months before slaughter. Holstein bull H500B became blind at 16 months of age but continued to produce large quantities of fair quality semen until slaughter. Examination of a testicle of this animal by biopsy *(1)* at 16 months of age showed reduced spermatogenesis and lack of cellular integrity in the seminiferous tubules. At slaughter, the testicles showed marked reduction of the interstitial tissue, the cells of Leydig being reduced in number and showing evidence of nuclear degeneration. The basement membrane of the seminiferous tubules appeared thickened and the Sertoli cells showed slight degenerative changes. There was reduced spermatogenesis. Figure 4 shows a specimen of the left testicle of Holstein bull H500B.

Jersey bull J221B showed similar damage to the testicles at slaughter but not so severe as bull H500B. The interstitial areas appeared normal. The biopsy specimen taken at 16 months was essentially normal. The adrenals, however, showed extensive damage (Figure 5). The capsule was thickened, the glomerulosa showed mucoid degeneration with numerous pyknotic cells and the reticularis was structurally normal except for a slight mononuclear infiltration extending from the medulla toward the periphery. The cells of the medulla showed pyknosis and mucoid degeneration. The adrenals of H500B and J200B1 showed similar but not as extensive degeneration.

The pituitary body of bull H500B was not available for examination, but that of bull J221B showed a few minute cysts in the pars nervosa and the pars distalis. A macroscopic cyst was found in the pituitary of bull H503B (Figure 6) together with cellular changes and destruction that resulted in an estimated

TABLE 2

Histolgical studies of organs and tissues of experimental cattle normal at birth but subjected to low carotene rations before and after birth

Experimental animal	Carotene intake of dam during gestation	Carotene intake of experimental animal		Observations and remarks
		Level	*Period*[a]	
	(γ/kg.)	*(γ/kg.)*	*(days)*	
Females				
J250	50 – last 3.5 months	50	45 – 969	Optic nerve, thyroid, and adrenals normal, microscopic pituitary cysts, inactive ovaries.
		90	969 – 1,364	
		260	1,364 – 1,865	
J252	50 – last 4.0 months	50	45 – 954	Optic nerve, thyroid, pituitary, adrenals normal, inactive ovaries.
		90	954 – 1,204	
		Normal	1,204 – 1,529	
J253	50 – last 4.0 months	50	45 – 934	Optic nerve, pituitary, thyroid, and ovaries normal, small cyst in right adrenal.
		90	934 – 1,300	
		130	1,300 – 1,327	
J255	50 – last 6.5 months	50	45 – 850	Constricted and degenerated optic nerve, pituitary cysts in pars nervosa, thyroid normal, ovaries inactive.
		90	850 – 1,355	
Males				
J218B	50 – last 7.0 months	50	45 – 498	Optic nerve, pituitary, thyroid, adrenals normal, reduced spermatogenesis.
H496B	50 – last 5.0 months	50	45 – 552	Optic nerve and thyroid normal, multiple minute pituitary cysts, reduced spermatogenesis.
J200B1	50 – last 2.8 months	50	45 – 612	Optic nerve, pituitary, and thyroid normal, partial invasion of medulla by cortex, reduced spermatogenesis.
		90	612 – 1,007	
		130	1,007 – 1,338	
J221B	50 – last 7.0 months	50	45 – 830	Optic nerve and thyroid normal, minute pituitary cysts in pars nervosa and pars distalis, adrenals show extensive mucoid degeneration, mononuclear infiltration, zona fasciculata reduced in size, cortex invading medulla, pyknosis, reduced spermatogenesis, slight degeneration of seminiferous tubules.
		90	830 – 1,225	
		130	1,225 – 1,437	
H557B1	130 – 280 da.	130	45 – 133	Optic nerve, thyroid, adrenals and testicle normal, multiple minute cysts in pars nervosa.
J267B1	130 – 275 da.	390	45 – 296	Optic nerve, thyroid, and adrenals normal, multiple minute cysts in pars nervosa, undeveloped testicle.
H500B	50 – last 6.0 months	50	45 – 876	Optic nerve constricted and degenerated, pituitary not available for examination, thyroid normal, some mucoid degeneration of adrenals with cortex invading medulla, reduced cells of Leydig, degeneration of seminiferous tubules, very little spermatogenesis.
		90	876 – 1,271	
		130	1,271 – 1,481	
H503B	50 – last 3.3 months	50	45 – 582	Optic nerve, thyroid and adrenals normal, large cyst on pituitary, only 15% functioning, cells of Leydig reduced, tissue debris in lumen of tubules.
		90	582 – 977	
		130	977 – 1,315	
J252B1	50 – 272 da.	50	45 – 90	Optic nerve and thyroid normal, cystic pituitary, mucoid degeneration of adrenals with cyst in zona fasciculata, reduced spermatogenesis, partial degeneration of the seminiferous tubules.
		90	90 – 485	
		130	485 – 1,028	

[a] Last day of experimental period gives age at slaughter.

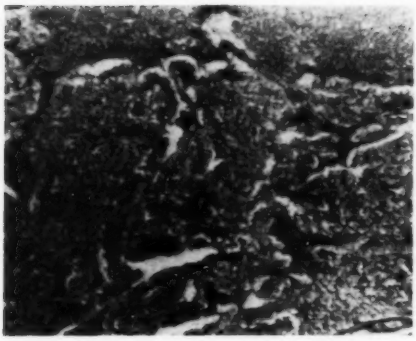

FIG. 5. Left adrenal gland of Jersey Bull 221B.

85% loss of functioning. Third generation bull J252B1 showed a cyst approximately $40 \times 350\,\mu$ involving the pars nervosa and the pars distalis (Figure 7). The adrenal gland of this bull showed small cysts in the zona fasciculata and mucoid degeneration of the medulla.

Four second generation low-carotene Jersey cows were slaughtered at 3 to 4 years of age, three because of failure to conceive and one because of acute mastitis. One cow (J250) showed microscopic cysts in the pars nervosa of the pituitary body, and another (J253) revealed a small cyst in the medulla of the

FIG. 6. Cystic pituitary of Holstein bull 503B.

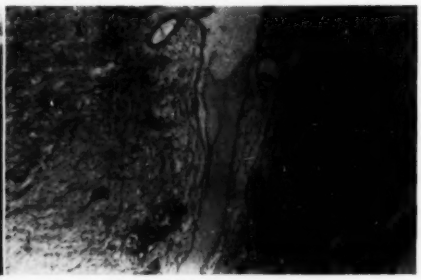

FIG. 7. Cystic pituitary of third generation Jersey bull 252B1.

right adrenal gland. Cow J255 which had gone blind at 16 months of age when receiving the $50\,\gamma$ of carotene per kilogram of body weight daily showed constriction and degeneration of the optic nerve. All four cows showed inactivity of the ovaries judging from the lack of developing follicles and the small number of corpora albicans. The other tissues appeared essentially normal.

DISCUSSION

The histological findings in this study are in general agreement with the findings of investigators who studied strictly deficient carotene rations *(3, 4, 5)*. Apparently $50\,\gamma$, $90\,\gamma$, and $130\,\gamma$ carotene per kilogram of body weight supplied

to second generation cows on suboptimal carotene intake will not entirely prevent degeneration of the optic nerves and development of cystic pituitaries. Calves born to these cows, even on a ration of 130 γ of carotene per kilogram of body weight per day, are apparently subject to disorders associated with low carotene feeding. It is possible that the long term exposure of the second generation animals to suboptimal carotene intake has detrimentally influenced a presently unknown conversion, utilization, or storage factor for carotene and/or vitamin A, with the result that the animal is no longer able to utilize the carotene supplied in the ration.

The condition of hydrocephalus encountered in five third generation low-carotene calves seems indicative that deficiency ration may exert some influence in producing this syndrome. Prior to the appearance of this condition in the experimental animals, there were no known cases of congenital internal hydrocephalus in the breeding herd from whence these experimental animals came. Records over a 25-year period reveal no mention of this condition. The possibility of hereditary influences can be discounted since members of the milking herd with similar ancestry and breeding have produced no hydrocephalic offspring.

In the second generation low-carotene bulls, degeneration of the seminiferous tubules, reduction of interstitial tissue and cells of Leydig, and reduced spermatogenesis were noted. Cystic pituitaries, showing both macroscopic and microscopic cysts, were seen. In three animals (J200B1, J221B1, H500B) a pathological condition of the adrenal gland was observed. Two third generation low-carotene bulls showed small cysts of the pituitary as early as 4 months and 10 months of age. The bull showing cysts at 4 months received 130 γ of carotene per kilogram of body weight per day and the other bull received 390 γ. This suggests that the continued exposure to suboptimal carotene intake influenced the physiology of carotene utilization to such an extent that neither 130 nor 390 γ of carotene per kilogram of body weight is sufficient to prevent tissue damage in the pituitary. It is further suggested that, since many of the calves showed damage of the optic nerve as well as of the pituitary gland, the prenatal influence on the fetus of suboptimal carotene intake by the dam is of some magnitude. Apparently the dam took care of her own needs for carotene before those of her fetus.

The appearance of a pathological condition of the adrenals in some animals suggests the possibility that vitamin A and/or carotene deficiency so affected the pituitary that the balance of hormones, particularly the adrenotropic hormone of the anterior pituitary, was altered enough to bring about degenerative changes in the adrenals.

Based upon the conditions of this study and the results obtained, it would appear that recommended vitamin A and carotene allowances for dairy cattle may require modification in the light of the duration of exposure. The data presented lead to the conclusion that long-time feeding of suboptimal or deficient-carotene rations has detrimental effects on the structures of certain tissues and organs, resulting in a loss of reproductive efficiency and viability.

SUMMARY

The feeding of suboptimum carotene rations for two or three generations resulted in abnormal reproductive behavior and often in damage to the pituitary, adrenal, and sex glands of the later generation animals.

Third generation calves dead at birth or blind, weak, and dying, or killed shortly after birth usually showed constriction and degeneration of the optic nerve and a hydrocephalic condition.

It would appear that the recommended carotene allowances for dairy cattle are too low if continuously fed beyond one generation.

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RELATIVE VALUE OF CAROTENE FROM ALFALFA AND VITAMIN A FROM A DRY CARRIER FED AT MEDIUM TO HIGH LEVELS TO HOLSTEIN CALVES[1]

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Using a group of forty-eight 63-day-old male calves, the authors set out to determine quantitatively the relative value of carotene from alfalfa and of vitamin A from a dry carrier, fed at medium to high levels. Blood and liver concentrations of vitamin A and vitamin A depletion time were used as criteria. *Editor.*

Both the minimum vitamin A and provitamin A, carotene, requirements of calves have been well established *(4, 14, 15)*. These have been based on intakes of vitamin A or carotene to maintain satisfactory growth or prevent the occurrence of night blindness, squamous metaplasia in the parotid gland or duct, papillary edema, or increased spinal fluid pressure. It has been recognized that these vitamin A or carotene requirements based on the above criteria do not permit appreciable vitamin A storage in the dairy calf. Although there was little concrete evidence in favor of feeding higher levels of vitamin A or carotene than necessary for normal growth or prevention of deficiency symptoms, the feeding of high enough levels of vitamin A and/or carotene to provide sufficient vitamin A storage has been indicated for the purpose of eliminating deficiencies during subsequent periods of low intakes of these substances and/or poor absorption due to scours *(5, 16)*. These were possibly some of the reasons that the National Research Council's Recommended Allowance *(11)* for carotene was approximately 2.0 to 6.0 times greater than the minimum requirements established for dairy calves on the basis of normal growth and prevention of deficiency symptoms.

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The present investigation was undertaken to determine quantitatively the relative value to the Holstein calf of carotene from alfalfa, and of vitamin A from a dry carrier, fed at medium to high levels, employing as criteria, primarily, blood and liver concentrations of vitamin A and vitamin A depletion time.

EXPERIMENTAL PROCEDURE

Animals and rations. Forty-eight 1-day-old male Holstein calves obtained from various Connecticut state institution herds during the period of September to December, 1954, were placed in individual tie-stalls at the University research barn. On arrival, each calf received a 500-mg. oblet of chlortetracycline[2] and 200,000 U.S.P. units of vitamin A in the form of fish liver oil.[3] Calves nursed their dams for 24 hours or received 8.0 lb. of colostrum during this period. Thereafter, each calf received approximately 174 lb. of milk to the 35th day of age, up to 3.0 lb. per day of a standard calf starter and ad libitum hay chopped to $\frac{1}{4}$ in. in length *(14)*. The hay graded on the average U. S. No. 1.3 alfalfa heavy grass mixed and had 61.3% color. The botanical composition was 1.2% foreign material with the remainder 57.0% alfalfa, 3.3% clover, and 39.8% grasses.

On the 64th day of age, each calf was fed an intake of a vitamin A depletion ration *(14)* to give an anticipated 7-day increase in live weight of 10 lb. This ration allowance was continued until each calf was slaughtered at the completion of the carotene or vitamin A feeding period or was removed from experiment at the completion of the final depletion period. When the blood plasma level of vitamin A for each calf had decreased to, or less than, 10 γ/100 ml., the depletion ration was supplemented with one of three levels of carotene from artificially dehydrated alfalfa leaf meal[4] or one of three levels of vitamin A from a dry carrier.[5] The carotene levels were 60, 180, and 540 γ per pound of live weight per day; the vitamin A levels were 5.5, 10.8, and 21.0 γ. The levels of carotene were chosen on the basis of the National Research Council Recommended Allowance *(11)* of 60 γ, and this level increased geometrically by a factor of 3 so that a range covering medium to high levels of carotene intake was insured. The levels of vitamin A were calculated from previous data *(14)* to give approximately equivalent responses to those anticipated for the chosen carotene levels. The first six calves to arrive at the research barn, replicate 1, were assigned to one of the six treatments according to a previously

[2] Lederle's Aureomycin, supplied by the Lederle Laboratories, Pearl River, N. Y., through the courtesy of Dr. Ralph Elliott.

[3] The fish liver oil contained 25% by weight of crude soybean lecithin and 25,000 U.S.P. units of vitamin A per gram. It was supplied by the Nopco Chemical Co., Harrison, N. J., through the courtesy of Dr. Melvin Hochberg.

[4] Artificially dehydrated alfalfa leaf meal was obtained from the National Alfalfa Dehydrating and Milling Co., Kansas City, Mo.; it contained no additives, such as antioxidants or oils.

[5] Dry carrier of vitamin A was obtained from the Nopco Chemical Co., Harrison, N. J. It was NOPCAY "10" Type IV from Lot No. V3587, a mixture of naturally occurring vitamin A esters on a dry carrier.

randomized allotment. This procedure was repeated until eight replicates of six calves each had been assigned to their respective treatments. The average age at the start of the carotene or vitamin A supplement feeding, the comparison period, was 78.2 ± 1.5 days. The duration of the comparison period was for each calf 12 successive 7-day age intervals.

At the completion of the comparison period, the calves in the even numbered replicates were slaughtered. Those in the odd numbered replicates were continued on the depletion ration without carotene or vitamin A supplement until each calf's blood plasma vitamin A level decreased to less than 4.0 γ/100 ml. for two consecutive 7-day intervals. This final depletion period was for the purpose of observing body stores of vitamin A *(10)*.

Calculation of the amount of supplemental carotene or vitamin A to be fed to each calf and its incorporation into the vitamin A depletion ration was identical to procedures used previously *(14)*.

Calves were exposed to artificial light only from 6 A.M. to 6 P.M. daily. Average intensity at a height of 48 in. in the center of each calf stall was 9.3 ± 3.3 foot-candles during the comparison period and 6.4 ± 3.3 during the final depletion period. Average daily minimum and maximum barn temperatures with their standard deviations were 55.0 ± 5.4° F. and 64.8 ± 7.0° F. for the comparison period and 60.0 ± 6.1° F. and 72.1 ± 6.4° F. for the final depletion period.

Observations and analyses. All feeds fed and refused were weighed to the nearest 0.1 lb. Live weights to the nearest pound were recorded on the seventh day of successive 7-day age intervals for each calf, as well as height at withers, heart girth, and circumference of barrel to the nearest ¼ in. at the beginning and completion of the comparison period. Venous blood samples for plasma carotenoids and vitamin A determination by the Kimble procedure *(3)* were obtained by puncture of the jugular vein on the fourth and seventh days of each calf's 7-day age intervals during the initial-depletion period and on the seventh day of successive 7-day intervals during the comparison and final depletion periods. Spinal fluid pressures *(12)* were observed during the last 7-day interval of the comparison period. From the calves slaughtered at the completion of the comparison period, the liver was removed for carotenoid and vitamin A *(8)*, dry matter *(2)* and lipid *(13)* analyses.

Samples of the feeds were taken at successive 4-week intervals during the course of the experiment. Samples of the artificially-dehydrated alfalfa leaf meal and of the dry carrier of vitamin A were taken at weekly intervals during all calves' comparison periods. These samples were analyzed for proximate constituents (Table 1) by A.O.A.C. procedures *(2)*; the alfalfa leaf meal for carotene by the official A.O.A.C. method *(2)* except extraction of the pigments employed the alternative extraction procedure of Method II, first action, in lieu of hot extraction; and the dry carrier for vitamin A by a modification *(1)* of the A.O.A.C. spectrophotometric method for fish liver oils *(2)*. The alfalfa leaf meal contained 0.246 ± 0.003 mg. of carotene per gram and the dry carrier, 2.951 ± 0.012 mg. of vitamin A (alcohol) per gram.

TABLE 1
Average chemical composition of feeds

	Per cent dry matter	Per cent of dry matter					
		Crude protein	Ether extract	Crude fiber	N.F.E.	Ash	Carotene *(mg/lb.)*
Alfalfa hay chopped	91.03 ± 1.08[a]	19.04 ± 0.26	2.30 ± 0.19	32.72 ± 0.28	37.38 ± 0.44	8.54 ± 0.19	17.28 ± 4.26
Calf starter	90.77 ± 0.95	19.94 ± 0.17	3.22 ± 0.26	6.07 ± 0.10	63.31 ± 0.60	7.43 ± 0.39	0.44 ± 0.14
Vitamin A depletion mixture	90.78 ± 0.73	15.65 ± 0.31	1.90 ± 0.10	13.62 ± 0.89	62.08 ± 0.90	6.72 ± 0.17	0.12 ± 0.02
Artificially dehydrated alfalfa leaf meal	94.26 ± 0.67	23.00 ± 0.36	3.03 ± 0.12	22.69 ± 0.96	37.79 ± 1.01	13.36 ± 0.20	111.63 ± 1.32
Dry carrier of vitamin A	95.09 ± 0.27	26.02 ± 0.25	37.38 ± 1.12	0.00 ± 0.00	33.51 ± 1.20	3.08 ± 0.06	— —

[a] Standard error of the mean.

Standard procedures *(6, 7, 17)* were employed in the statistical analyses of the data.

RESULTS AND DISCUSSION

Feed consumption, growth, and health. Calves readily consumed their allotted amounts of depletion ration or depletion ration plus carotene or vitamin A supplement. Thus weigh-backs, which averaged 0.9 days per calf during the comparison period, were not a problem in this study nor were they related to treatment.

The average weight of the calves at the start of the carotene or vitamin A supplementation, comparison period, was 190 lb., which had a standard deviation of 22 lb.; height at withers, 34.2 ± 1.1 in.; heart girth, 37.7 ± 1.9 in.; and circumference of barrel, 46.3 ± 2.9 in. During the 12-wk. comparison period, the average increase in live weight was 148 ± 13.5 lb.; in height at withers, 5.3 ± 0.7 in.; in heart girth, 8.4 ± 1.5 in.; and in circumference of barrel, 10.9 ± 2.7 in. Differences among calves fed different levels of carotene or vitamin A or between calves fed either carotene or vitamin A were inappreciable and upon statistical analyses were found not to be related to treatment.

The percentage days free of scours during the comparsion period, 99.6% (mean of the arc sin $\sqrt{\%}$ days free of scours, 86.62 ± 5.32, transformed back to percentage) and the number of treatments necessary when the rectal temperature was 103° F. or greater, 0.2 (mean of the $\sqrt{\text{No. of treatments} + \frac{1}{2}}$, 0.81 ± 0.21, squared minus one-half) were not related to treatment. The average spinal fluid pressure at the completion of the comparison period was 96 ± 9 mm. H_2O. Of possible interest was the observation that the magnitude of the spinal fluid pressure tended to be slightly greater in the calves receiving the lowest intakes of vitamin A or carotene. It should be noted, however, that pressures below 90 mm. of water were not measurable by the method used *(12)*.

Plasma and liver carotenoid and vitamin A concentrations and vitamin A depletion time. Blood plasma carotenoids (Table 2) increased at diminishing

TABLE 2

Effect of level of carotene or of vitamin A intake on plasma carotenoids and plasma vitamin A of Holstein calves

	Plasma carotenoids[a]			Plasma vitamin A[a]	
		Comparison period			Comparison period
	Initial	Actual	Log[b]	Initial	Actual
	(γ/100 ml. plasma)				
Carotene intake					
60 γ	4	32	1.614	8.6	18.7
180 γ	10	63	1.846	7.1	22.9
540 γ	5	111	2.044	8.6	27.2
Vitamin A intake					
5.5 γ	7	2	1.085	9.6	12.5
10.8 γ	7	2	1.065	7.9	17.4
21.0 γ	4	1	1.023	8.9	25.3
Standard deviation of a calf	4	—	0.081	1.7	3.2
Rate of linear change per log-log *(10 times γ intake)*					
Carotene	—	—	3.3636 $\pm$0.3166[c]	—	—
Vitamin A	—	—	$-$0.4904 $\pm$0.3213	—	—
Rate of linear change per log *(γ intake)*					
Carotene	—	—	—	—	8.8588 $\pm$ 1.7035[c]
Vitamin A	—	—	—	—	22.0169 $\pm$ 2.7822

[a] Eight calves per intake group, data for the comparison period during which carotene or vitamin A was fed.

[b] Log (γ/100 ml. plus 10).

[c] Standard error of linear rate of change, s_b.

rates with increasing carotene intakes from artificially dehydrated alfalfa, and, as expected, slight but inappreciable depression occurred in plasma carotenoids with increasing vitamin A intake. Blood plasma vitamin A also increased at diminishing rates with increasing carotene or vitamin A intake. Liver carotenoids, liver vitamin A, and vitamin A depletion time (Table 3) increased proportionally with an increase in level of carotene intake from artificially dehydrated alfalfa. Liver carotenoids decreased and liver vitamin A and vitamin A depletion time increased arithmetically with increasing intake of vitamin A from the dry carrier. Inappreciable differences among levels of carotene or vitamin A or between carotene and vitamin A were observed in liver dry matter or liver lipids.

The variances of plasma carotenoids, liver carotenoids, and liver vitamin A concentrations tended to increase with increasing intake of carotene or vitamin A. Transformation of these criteria to their respective logarithms (base 10) resulted in minimal differences among the variances of the intake groups. This

TABLE 3

Effect of level of carotene or of vitamin A intake on liver carotenoids, liver vitamin A, and vitamin A depletion time of Holstein calves

	Liver[a]							Vitamin A[b] depletion time
	Weight	Dry matter	Lipids	Carotenoids Actual	Carotenoids Log	Vitamin A Actual	Vitamin A Log	
	(g.)	*(g/100 g.)*		*(γ/100 g.)*				*(wk.)*
Carotene intake								
60 γ	2552	27.5	3.9	96	1.979	287	2.446	4.4
180 γ	2616	27.9	3.8	115	2.049	748	2.846	6.9
540 γ	2619	27.7	3.8	226	2.338	1833	3.235	12.6
Vitamin A intake								
5.5 γ	2510	27.4	4.0	26	1.318	101	1.980	2.8
10.8 γ	2426	28.0	3.9	22	1.268	500	2.683	4.4
21.0 γ	2582	27.7	4.0	28	1.388	1380	3.108	10.3
Standard deviation of a calf	193	0.6	0.3	—	0.207	—	0.174	1.0
Rate of linear change per γ intake								
Carotene	—	—	—	—	—	—	—	0.0166 ± 0.0014[c]
Vitamin A	—	—	—	—	—	—	—	0.4955 ± 0.0465
Rate of linear change per log *(γ intake)*								
Carotene	—	—	—	—	0.3768[c] ±0.1534	—	0.8276 ±0.1293	—
Vitamin A	—	—	—	—	0.1196 ±0.2515		1.9410 ±0.2120	—

[a] Four calves per intake group, data for calves slaughtered at the completion of the carotene or vitamin A feeding period.

[b] Four calves per intake group; data for calves not slaughtered and fed the depletion ration until the blood plasma vitamin A level decreased to less than 4 γ/100 ml. for two consecutive 7-day periods *(10)*.

[c] Standard error of linear rate of change, s_b.

confirmed a previous report *(9)*, which dealt with a similar problem in dairy calves in which a logarithmic transformation was indicated *(6, 7, 17)*. Therefore, in the analysis of variance of these data, the logarithms were used so that the condition of common variance among intake groups would be met *(6, 7, 17)*.

Another problem in the statistical treatment of the data was selection of appropriate methods of obtaining linear relationships of the response criteria to intake. Since it has been quite well recognized that the magnitude of the response of either plasma carotenoids or vitamin A concentrations diminished as intake of carotene or vitamin A increased, these criteria were fitted on the logarithms of the intake. This resulted in essentially linear relationships. Where log plasma carotenoid values were indicated, the fitting of these transformed response criteria on log-log carotene intake (or on log-log vitamin A intake) resulted in linear relationships. Similarly, when log liver carotenoids or log liver vitamin A concentrations were used, the fitting of these criteria on log carotene or log vitamin A intake maintained the linear relationships. These relationships are given at the bottom of Tables 2 and 3. In the log (plasma carotenoids + 10),

99.99% of the variance attributable to within-carotene levels and 88.62% attributable to within-vitamin A levels was accounted for by the indicated functions under the conditions of this experiment. Similar values for plasma vitamin A were, respectively, 99.99% and 98.04%; for log liver carotenoids, 88.99% and 33.34%; for log liver vitamin A, 99.99% and 97.84%; and for vitamin A depletion time, 97.90% and 99.65%.

Relative values of carotene and vitamin A. In Table 4 are given the amounts

TABLE 4

Relative value of carotene from artificially dehydrated alfalfa and vitamin A from a dry carrier

Amount of carotene from artificially dehydrated alfalfa fed	Amount of vitamin A from a dry carrier necessary to obtain equivalent response to carotene	Ratio of carotene to vitamin A
(γ/lb. live weight/day)		*(γ carotene/γ vitamin A)*
Based on plasma vitamin A[a]		
60	11.17	5.37
180	17.37	10.36
540	27.03	19.98
Based on liver vitamin A[b]		
60	9.91	6.05
180	14.43	12.47
540	23.05	23.43
Based on vitamin A depletion time[c]		
60	10.06	5.96
180	14.08	12.78
540	26.14	20.66

[a] From the relationship $x_2 = 0.3325 + 0.4024x_1$ in which x_2 = log (γ vit. A/lb. live weight/day) and x_1 = log (γ carotene/lb. live weight/day) from data in Table 2.

[b] From the relationship $x_4 = 0.1978 + 0.4264x_3$ in which x_4 = log (γ vit. A/lb. live weight/day) and x_3 = log (γ carotene/lb. live weight day) from data in Table 3.

[c] From the relationship $x_6 = 8.062 + 0.0335x_5$ in which x_6 = (γ vit.A/lb. live weight/day) and x_5 = (γ carotene/lb. live weight/day) from data in Table 3.

of vitamin A necessary to obtain equivalent responses to 60, 180, and 540 γ carotene intakes based on plasma and liver vitamin A concentrations and vitamin A depletion time.[6] Based on these three response criteria, carotene on a weight basis was equivalent to between 1/5th and 1/7th of that for vitamin A alcohol when carotene was fed at the 60 γ intake level, between 1/10th and 1/13th for the 180 γ intake level and 1/20th to 1/24th for the 540 γ intake level. These equivalencies extend previous findings, as recently reviewed *(14)*, to ranges of carotene and vitamin A intakes which provide storage of vitamin A.

[6] These equivalencies were obtained from the response criteria in Tables 2 and 3. For example, plasma vitamin A, y, on log (carotene intake), x_1, resulted in the linear function 1.) $y = 2.92 + 8.8588x_1$ and on log (vitamin A intake), x_2, resulted in the linear function 2.) $y = -4.40 + 22.0169x_2$. Now letting y in 1.) equal y in 2.) and solving for x_2, equation 3.) $x_2 = 0.3325 + 0.4024x_1$. This gives log (vitamin A intake) as a function of log (carotene intake). By substituting appropriate values for carotene intake, x_1, values for vitamin A intake, x_2, then can be determined.

Therefore the data give information for ranges of carotene or of vitamin A intakes which are likely to be used in practice and include equivalencies for the National Research Council's Recommended Allowance *(11)* for carotene of 60 γ per pound live weight per day.

SUMMARY

Forty-eight 63-day-old male Holstein calves, previously fed limited whole milk to 35 days of age and up to 3.0 lb. of starter and ad libitum chopped alfalfa hay, were fed an intake of a vitamin A depletion ration to give an anticipated 10 lb. increase in live weight per 7-day period. When the blood plasma vitamin A level for each calf decreased to 10.0 γ/100 ml. or less, each calf was fed in addition to the depletion ration one of three levels of carotene, 60, 180, and 540 γ from alfalfa, or one of three levels of vitamin A, 5.5, 10.8, and 21.0 γ from a dry carrier, daily per pound of live weight for 12 successive 7-day age periods. Upon the completion of the supplement feeding period half of the calves were slaughtered. The remaining calves were then maintained on only the depletion ration until the blood plasma vitamin A level decreased to less than 4 γ/100 ml. for two consecutive 7-day periods for the purpose of observing vitamin A depletion time. The following results were obtained:

1. Plasma carotenoids and vitamin A concentrations increased at diminishing rates with an increase in the carotene intake from artificially dehydrated alfalfa. Liver carotenoids and vitamin A concentrations as well as vitamin A depletion time increased proportionally with increased carotene intake.

2. When the calves received vitamin A intake from a dry carrier, inappreciable differences were observed for plasma and liver carotenoid concentrations. Plasma vitamin A increased at diminishing rates and liver vitamin A and vitamin A depletion time at constant rates with increased vitamin A intake.

3. From the relationships of plasma and liver vitamin A concentrations and vitamin A depletion time with carotene or vitamin A intake, it was found that on a weight basis carotene fed at the 60 γ intake level was equivalent to approximately 1/5 to 1/7th as much as vitamin A (alcohol); at the 180 γ intake level, 1/10th to 1/13th; and at the 540 γ intake level, 1/20th to 1/24th.

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THE EFFECT OF GLYCEROL LEVEL AND RATE OF FREEZING, FOR VARIOUS EXTENDERS, ON THE SURVIVAL OF BOVINE SPERMATOZOA FROZEN AND STORED AT −79° C.[1]

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With three different extenders the optimum levels of glycerol and rates of freezing were determined for the semen from a Holstein bull for a period sufficient to obtain ten usable collections. A 7% glycerol level, the use of a chemical–milk extender, and a freezing rate of 2.2°-3.3° C. per minute were found to produce superior results. *Editor.*

Much recent research *(4, 8, 11, 12)* has been conducted to determine the optimum conditions for freezing bovine semen. However, many questions remain unanswered.

Since glycerol has been found to be essential to the survival of spermatozoa when frozen, many experiments have been initiated to determine the optimum level. Smith and Polge *(13)* reported a level of 10-15% as giving good survival. Later, Polge *(10)* reported 10% as being more desirable. More recent workers *(7, 8, 16)* have reported levels of less than 10% as being satisfactory. Different levels have been reported for different extenders *(6, 9)*.

In experiments comparing rates of freezing, Smith and Polge found that when semen was cooled in 14 stages of 2.5 minutes each, better survival was obtained than when cooled at a rate of 1° C. per minute. Graham *et al. (6)* found the optimum rate to be 2° C. per minute to −30° C. and 5° C. per minute from −31° C. to −79° C. when heated whole milk was used as an extender. Erickson *et al. (5)* used a cooling rate of 3° C. per minute, and O'Dell and Almquist *(9)* used slower rates of cooling. Williams *(17)* reported his best freezing rate as 1° C. per minute down to −15° C. and then at a faster rate to −79° C.

Although different combinations of glycerol levels and rates of freezing for different extenders have been compared by Dunn and Hafs *(3)* and by Erickson *et al. (5)*, the optimum combinations of these variables have not been definitely established. The experiment reported here was initiated to determine the optimum levels of glycerol and rates of freezing for three extenders.

EXPERIMENTAL PROCEDURE

Semen used in this experiment was collected from a Holstein bull once weekly for a period of time sufficient to obtain 10 usable collections. First and second ejaculates were pooled in order to have a sufficient quantity of semen.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Routine laboratory examinations were made, and only samples with initial motility of 50% or above were used.

Semen suitable for processing was extended at the rate of 1:25 in one-half the final volume of each of the three extenders and cooled to 5° C. in 4 hours. The yolk-citrate extender was composed of one part egg yolk to four parts 2.9% sodium citrate. The heated milk was prepared by the method of Thacker and Almquist *(15)*. In the preparation of the chemically treated milk extender a 70% solution of thiovanic acid[2] was diluted 1:100 with 2.9% sodium citrate. One ml. of this stock solution was added to each 50 ml. of milk.

Glycerol was added to one-half of each extender at the rate of 14, 20, and 30% by volume and allowed to cool to 5° C. over a period of 4 hours. After cooling, the glycerolated portion of each extender was added to the part containing semen in five equal parts at 6-minute intervals. This gave a final dilution rate of 1:50 and glycerol levels of 7, 10, and 15%.

The glycerolated semen was allowed to equilibrate for a period of 18 hours. All syringes used in filling and the 1.6-ml. glass ampules were placed in the refrigerator at this time so that all equipment coming in contact with the semen would be the same temperature as the semen.

A total of 270 ampules, 10 for each treatment, were filled from each collection and sealed with an oxygen–natural gas burner. Each glycerol level within each extender was divided into three freezing rates of 1.1, 2.2, and 3.3° C. per minute. Acetone was used as the freezing medium, being cooled by manual addition of cracked dry ice. After a temperature of –34.4° C. was reached, all samples were frozen at the rate of 4.4° C. per minute to –75° C. and then placed in trays containing acetone in a dry ice storage box.

Evaluation of the percentage of motile sperm in the extended semen after being frozen and stored for 2 weeks was based on the average of two readings from each of three ampules from each treatment. The ampules were thawed at 5° C. Snedecor's *(14)* methods were used in determining the statistical significance of the treatment differences.

RESULTS AND DISCUSSION

Table 1 shows the survival percentage after 2 weeks storage of each of the 27 treatment combinations. The survival was significantly influenced by glycerol level, rate of freezing, extenders, and collections.

The survival percentage for the treatments containing 15% glycerol were so extremely low, averaging 3.3%, that those data were omitted in the analysis of variance. As shown in Table 1, the 7% glycerol level averaged 76.9% survival compared to 70.6% survival for the 10% level. The difference was highly significant. It is of interest that the 7% level gave a higher survival for the yolk-citrate and heated homogenized milk extenders, whereas the 10% level was superior in the chemical milk extender.

The three freezing rates of 1.1, 2.2, and 3.3° C. per minute gave average survival rates of 69.5, 76.5, and 75.5%, respectively. The slowest freezing rate

[2] Vacuum distilled thioglycolic acid available from Evans Chemetics Co.

TABLE 1

Percentage survival of spermatozoa, by treatments, after 2 weeks storage at −79° C. (Average for 10 collections)

Glycerol level	Freezing rate per minute	Extenders			Average	
		Chemical milk	Heated milk	Yolk citrate	Freezing rate	Glycerol
		(%)	(%)	(%)	(%)	(%)
7%	1.1° C.	79.2	75.7	70.5	75.1	
	2.2° C.	83.0	78.0	74.9	78.6	76.9
	3.3° C.	84.5	78.3	69.0	77.2	
	1.1° C.	78.1	65.4	48.1	63.8	
10%	2.2° C.	85.1	70.5	67.7	74.4	70.6
	3.3° C.	84.6	73.3	63.5	73.8	
Average		82.4	73.5	65.6		
	1.1° C.	0.6	0.5	0.6	0.5	
15%	2.2° C.	0.9	4.6	7.1	4.2	3.3
	3.3° C.	0.7	6.5	6.8	4.6	
Over-all average		55.2	50.3	45.4	50.3	

was significantly lower than the two other rates ($P < 0.01$). No significant difference was found between the 2.2 and 3.3° C. per minute rates.

Chemically treated milk extender resulted in a survival rate of 82.4%, whereas the value for heated homogenized milk was 73.5% and for yolk-citrate was 65.6%. These differences were highly significant. However, as mentioned previously, the values for 15% glycerol were not included. When the 15% glycerol treatments are included, the survival percentage is lower, but the extenders remain in the same order of desirability.

In this experiment, with collections corresponding to replicas or trials, a highly significant difference was found between the collections from the same bull taken on successive weeks. However, some variation in the freezability of the various ejaculates was expected. Brugman and Poore *(2)* had previously demonstrated a difference among breeds, bulls within herds, and also ejaculates from the same bull. In this experiment the survival percentages for individual ejaculates over all freezing rates, all extenders and the 7% and 10% glycerol levels varied from 56.5 to 84.4, the average of the ten collections being 74.3.

Of the interactions studied, only those between glycerol level and extender and collection and extender were found to be significant. The interaction of collection and glycerol level approached significance at the 5% level.

SUMMARY

In a study of sperm survival in three different extenders containing three levels of glycerol and frozen at three different rates it was found that a 7% glycerol level resulted in significantly higher survival percentage than either a 10% or 15% level. The 15% level was in no way desirable. A chemical-milk extender, with an average survival percentage of 82.4, was superior to heated homogenized milk or yolk-citrate. A freezing rate of 2.2° C. or 3.3° C. per minute gave significantly higher survival percentages than a rate of 1.1° C. per minute.

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GESTATION LENGTH IN AN INBRED JERSEY HERD

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From the records of 427 inbred Jersey cows since 1921 a study was made of the nature and causes of variation in gestation lengths. The partitioning of genetic from environmental variation and the effects of inbreeding and milk production are given particular attention. *Editor.*

The length of a gestation period is determined by numerous factors acting and interacting in an intricate pattern. Many studies have dealt with the average length of gestation in cattle as it is affected by such factors as breed, family, sex of calf, twinning, age of dam, and season. Studies that attempt the quantitative separation of genetic and environmental factors are less numerous. Among the latter are the publications of Düring *(8)*, Jafar *et al. (14)*, and Brakel *et al. (2)*. The last reference includes an extensive bibliography on the duration of gestation in cattle.

The present study was undertaken to obtain further information about the nature and causes of variation in gestation lengths; the partitioning of genetic from environmental variation and the effects of inbreeding and milk production are given particular attention.

SOURCE OF DATA

The data for this study are the records of 1,353 gestation periods of 427 cows of the University of California inbred Jersey herd. The gestation periods used were restricted to those ranging from 260 to 301 days and terminating with the birth of a single, live calf. The data were gathered from 1921 to date. The section of the study dealing with the relationships between first lactation and second gestation includes the records of 171 cows.

The general mating plan in this herd has been to mate sire to daughter for as long as the sire continued in service and to replace him with a progeny-tested son, preferably an inbred son. Deviations from this scheme were necessary, since ideal replacement sires were not always available. Although the 427 cows represent the daughters of 48 sires, 22 of the sires account for over seven-eighths of the data. Four lines were started, but two were later sold because of a shortage of funds; finally, the remaining two were merged to form a single closed line. In 1946, three bulls related to each other, but unrelated to the herd, were introduced. Their outcross progeny were used to start a second line related to the

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remaining original line. Complete genetic covariance charts have been constructed for the herd. Inbreeding coefficients ranged from 0 to 62% and averaged 19%.

A conscious effort has been made throughout the experiment to maintain as constant an environment as possible from birth through the first lactation. The management practices have remained reasonably constant. The only major shift was a gradual one from three-times and four-times milking to two-times in the late 1930's. The plane of nutrition has been kept constant; during periods of short funds, numbers have been reduced rather than reduce the feeding plane. Only single lactation records under reasonably constant environment are available for the majority of the cows. Nearly all cows were entered on Register of Merit Test for their first lactations. Pasture time has always been restricted on the Official Test string in an attempt to reduce variation due to good and poor pastures and pasture seasons. Roughage intake has been controlled in order to assure a high concentrate intake. On completion of the Official Test record, cows are available for use in any experiment or demonstration calculated not to interfere seriously with reproductive performance. The plane of nutrition is nearly always lower for second and later lactations. Full use is made of available pasture in season and maximum roughage intake is encouraged, concentrate feeding being somewhat restricted.

ANALYSIS

Mean length of gestation. The frequency distribution of individual gestation period lengths for the 1,353 records used in this study is presented in Figure 1. These records are uncorrected for sex or calving sequence. The mean length of the uncorrected records is 282.7 ± 0.15 days with a standard deviation of 5.4 days. The distribution of cows according to the number of gestation records they contributed to this study is given in Table 1. The mean was three gestations per cow.

Brakel *et al. (2)* point out that in published studies a uniform procedure has not been used to calculate the number of days in the gestation period. The method

Figure 1.

THE DISTRIBUTION OF 1,353 NORMAL, SINGLE-BIRTH GESTATION PERIODS, UNCORRECTED FOR SEX OR CALVING SEQUENCE.

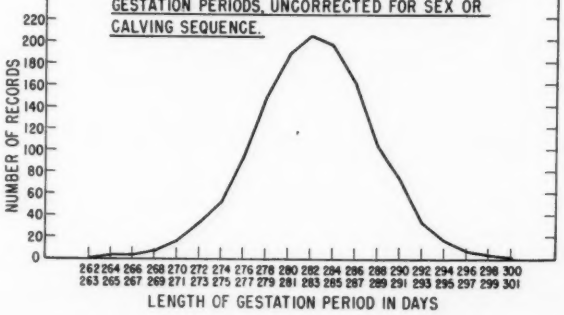

TABLE 1

The percentage distribution of the 427 cows according to the number of gestation records they contributed

	Number of gestation records per cow							
	1	2	3	4	5	6	7	8 to 13
Percentage of cows in each gestation frequency class	24	23	17	14	7	7	5	3

used here is that of the above authors, i.e., the day the calf was born is counted but not the day of last service. Brakel *et al.* list nine studies in which the average gestation period for the Jersey is reported to be 277.9 to 284.3 days. For their own study, based on 249 records, they report an average length of 277.9 ± 0.38 days with a standard deviation of 5.9 days. The difference of 4.8 days between the above-mentioned study and the present one has a standard error of ± 0.41 days, an indication that the two samples are not from the same population of cattle. Only two other studies report average gestation lengths of over 280 days for the Jersey. Fitch *et al. (9)* and Herman *et al. (11)* reported means of 284.3 and 280.4 days, respectively. Fitch *et al.* included both the day of service and the day of birth in calculating average length, but Herman *et al.* did not include the day of service. Copeland *(3)* gives what is probably the best estimate of gestation length for the Jersey breed. His sample of 1,075 gestation records came from calving affidavits sent to the cattle club after the completion of Register of Merit records. Probably a fairly large number of herds is represented, and all cows must have met the minimum production entry requirements. The average gestation length was 278.51 days. His method of calculating length is that of the present study. His data included periods ranging from 228 to 312 days. The elimination of the 19 records in Copeland's study that were under 260 days would not raise the average gestation length over 279 days. The differences between the averages discussed above are similar to those found by Ward and Castle *(37)*, who report significant differences in gestation lengths of dairy cattle between three districts of New Zealand. Ward and Castle, however, make no mention of breed.

Sex and calving sequence. Variations in sex of calf and age of dam as measured by calving sequence were studied as possible contributing sources of variation in gestation length. Calving sequence was classified into seven classes starting with "1" and ending with "7 and up." Calving sequence 1 refers to a cow's first calving, calving sequence 2 to second calving, etc. By using the method of expected subclass numbers *(32)* the following analysis of variance was made:

Source of variation	*Degrees of freedom*	*Mean square*
Sex	1	1,181.93**
Calving sequence	6	211.53**
Sex × calving sequence	6	24.21
Within subclasses	1,082	26.14

**Significant at the 1% level.

The analysis yielded the following correction factors. Male calves were carried 2 days longer than female calves. First calves were carried 1.3 days less than second calves; second calves were carried 1.2 days less than third calves. There were no significant differences nor was there any trend among calving sequences 3 and up. The age of cow at first calving was about 26 months.

Year and season. Some information regarding possible year and season effects on gestation length was considered necessary. To avoid confounding sire differences with such effects, an analysis on an intrasire basis was carried out. The sire with which the largest number of data was associated was chosen for the analysis. This sire produced 204 offspring during a 6-year period. The seasons used were the four conventional calendar seasons. Prior to this analysis the data were adjusted for sex and calving sequence effects. In a least squares analysis the linear model contained variables for year, season, year × season interaction, inbreeding of calf, and inbreeding of dam.

Year × season interaction was significant at the 1% level of probability. Since interaction proved significant, the main effects were tested in an analysis by using the method of weighted squares of means (Snedecor, *31*). This method gives unbiased estimates and tests of the main effects if interaction is present. Neither year nor season effects proved significant. The F values in each case were less than unity. The results of the analysis imply the existence of short-term time trends not associated with season. No appropriate method of adjusting the data for such effects is apparent.

In addition to the above analysis, which dealt with only the offspring of a single bull, all of the data were analyzed for possible month and season effects. Monthly and seasonal averages are presented in Table 2. As previously stated, these data were adjusted for sex and calving sequence to a male calf–mature dam equivalent. The analysis of variance presented in Table 3 indicates that seasonal differences do not contribute to variation in gestation length.

TABLE 2

Average gestation length according to month and season of calving[a]

Month	Number of calvings	Monthly average	Seasonal average
		(*days*)	(*days*)
December	99	285.0	
January	117	284.8	284.6
February	133	284.3	
March	150	285.0	
April	119	284.0	284.5
May	114	284.3	
June	118	284.0	
July	110	284.9	284.2
August	100	283.8	
September	100	285.0	
October	115	284.4	284.7
November	78	284.6	
Total	1,353		284.5

[a] Gestation lengths adjusted to a male calf, mature dam equivalent.

TABLE 3
Analysis of variance of gestation length for month and season of calving

Source of variation	Degrees of freedom	Mean square
Seasons	3	5.79
Months within seasons	8	26.57
Within months	1,341	27.43

Inbreeding. A study was made of the relation of inbreeding of calf and inbreeding of dam to gestation length. Data from 1,236 calves sired by 22 bulls were analyzed. The inbreeding of the calves and of their dams is distributed as follows:

Coefficients of inbreeding (in percentages)	0 to 6	7 to 13	14 to 21	22 to 28	29 to 35	36 to 42	43 to 49	50 to 56	57 to 63
Percentage of calves	31.4	12.9	12.6	18.8	9.0	8.5	4.6	1.6	0.5
Percentage of dams	52.8	14.1	10.8	13.5	3.9	3.1	1.1	0.6	0

The average inbreeding of the calves is 18.7% and that of their dams is 10.5%. The latter average is weighted according to the number of calves a dam produced.

Intrasire partial regression coefficients of gestation length on inbreeding of dam and on inbreeding of calf were calculated. The model used was:

$$Y_{ij} = \mu + b_i X_{ij} + c_i Z_{ij} + e_{ij}$$

where Y_{ij} = gestation length associated with the jth calf of the ith sire,
μ = an effect common to all the gestation lengths,
X_{ij} = inbreeding of the dam of the jth calf of the ith sire,
Z_{ij} = inbreeding of the jth calf of the ith sire, and
e_{ij} = the difference between the observed and expected gestation length of the jth calf of the ith sire.

The following hypotheses were tested:

Hypotheses tested	*Sample F*	*Critical levels of F*		*Comments*
		F 0.05	F 0.01	
$b_i = c_i = 0$	1.70	1.39	1.59	Hypothesis rejected
$c_i = 0$	1.38	1.55	1.85	Hypothesis not rejected
$b_i = 0$	1.95	1.55	1.85	Hypothesis rejected
$b_i = b$	3.15	1.55	1.85	Hypothesis rejected

The common intrasire partial regression coefficients of gestation length on inbreeding of dam and on inbreeding of calf, respectively, are $b = 0.029$ and $c = 0.020$. The fact that b is positive shows that in general the more inbred the dam is, the longer she carries her calf. However, the rejection of the hypothesis $b_i = b$ implies that the magnitude of the effect of inbreeding of the dam varied significantly among the groups of dams mated to the 22 sires.

Partitioning of variance. In partitioning the phenotypic variance of a trait into genetic and environmental components of variance, it is necessary to set up various sorts of families, e.g., paternal half-sib families, maternal half-sib families, full sibs, etc. More generally for the purpose of genetic analysis a family may be quantitatively described by the average genetic relationship between members of the group called a family. In a random breeding population, or in one divided into distinct unrelated inbred lines, the various intra- and interclass correlations associated with the different types of families can, with the knowledge of the relationship within the families, be used in deriving estimates of the genetic and environmental components of phenotypic variance.

The data in the present study are obtained from an inbred herd in which family lines are far from distinct; that is to say, families of paternal half-sibs are related in varying degrees to each other. The same holds true for maternal half-sib and full-sib families. The effective genetic relationship (r) between members of the same family in such a situation is $\frac{r_2 - r_1}{1 - r_1}$, where r_2 is the relationship within the family and r_1 is the relationship between families (Lush, *25*). To find r_1 in this case would be difficult. It was therefore decided to work with pooled estimates of variance within families. Table 4 presents these estimates along with their theoretical composition.

For each type of family, a, the coefficient of the G term is $(1 + f - cov)$ where f = average inbreeding of calves in the family and cov is the average covariance (the numerator of the coefficient of genetic relationship) between family members.

TABLE 4

Pooled estimates of within family variances and their theoretical composition

Type of family	Total number of calves involved	Degrees of freedom for σ^2	Pooled estimate of within family variance σ^2		Theoretical composition of within-family variance[a] $a\,G + E + \beta\,G_D + M$
Paternal half-sib	950	760	20.89	=	$0.60G + E + 0.68\,G_D + M$
Maternal half-sib	332	166	18.24	=	$0.65G + E$
Full sib	728	364	16.26	=	$0.44G + E$
Non-sib	970	776	26.34	=	$0.96G + E + 0.89G_D + M$

[a] G = Component of variance due to additive genetic differences between calves.
G_D = Component of variance due to additive genetic differences between dams.
M = Component of variance due to permanent differences between dams other than those included in G_D.
E = Component of variance due to unanalyzed residual effects.

The coefficient of the G_D term, β, is $(1 + f' - cov')$ where f' = average inbreeding of the dams producing the calves of a family and cov' is the average covariance between such dams. Dickerson *(7)* discusses the theory relevant to deriving coefficients of this type.

The manner in which the equations in Table 4 were obtained is illustrated below for the case of paternal half-sib families.

Family	Estimated within family variance	Theoretical composition of within family variance
1	$\sigma 1^2$	$a_1 G + E + \beta_1 G_D + M$
2	$\sigma 2^2$	$a_2 G + E + \beta_2 G_D + M$
3	$\sigma 3^2$	$a_3 G + E + \beta_3 G_D + M$
•	•	•
•	•	•
•	•	•
i	σi^2	$a_i G + E + \beta_i G_D + M$
•	•	•
•	•	•
•	•	•
N	σN^2	$a_N G + E + \beta_N G_D + M$

$$\sigma^2 = \frac{\sum_{i=1}^{N} \sigma i^2}{N}$$

$$a\,G + E + \beta G_D + M = \frac{\sum_{i=l}^{N} ai\,G}{N} + E + \frac{\sum_{i=l}^{N} \beta i\,G_D}{N} + M$$

All families used within a given type of family had equal numbers. This qualification was felt desirable since it was necessary to average the σi^2 and the ai and βi. If the families had varied widely in number, it would have been difficult to weight the ai and βi properly in the averaging process since their distribution functions are not known. In paternal half-sib families the sire family used contained five members. A paternal half-sib family larger than five was divided into families of five by random selection. Non-sib families also contained five members. Non-sib refers to a family of calves in which no two calves have a parent in common. Full-sib families and maternal half-sib families contained two members each. The family sizes chosen enabled the use of the most data subject to the restriction that all families of the same type have equal numbers.

The solution of the four simultaneous equations in Table 4 is $G = 9.43$, $E = 12.11$, $G_D = 9.81$, and $M = -3.55$. Since a variance cannot be negative, our best estimate of M is that it equals zero.

Interrelationships between gestation length and performance. The interrelationships studied were those between the duration of gestation and FCM yield, body weight, body weight change during lactation, length of dry period, length of period from service to conception, and length of the period during which lactation and gestation overlapped. There were 171 cows whose records included the following necessary data: *(a)* monthly body weight records, *(b)* an ROM test in the first lactation, and *(c)* a normal second gestation. Gestation length was corrected to a male basis. These cows calved at an average age of 26.5 months for their first lactation. This part of the study deals with data pertaining to the first lactation and the second gestation.

One objective was to determine to what extent the work a cow did during lactation might affect the length of the gestation period. Milk production, the major energy expenditure, was expressed on the constant-energy basis of FCM *(10)*. Records were not adjusted for frequency of milking, which was two times and three times, or for age at first calving. The majority of the records covered 305 days; shorter records were not extended, but a number of longer records were cut off at 305 days. The average production for the sample was 9,136 lb. of FCM. Body weight was estimated from the monthly weight taken just before first calving; this averaged 915 lb. Body weight change was estimated by the slope of the line fitted to the six monthly body weights following the first full month after calving. The average monthly change was a gain of 11 lb. In calculating the length of time that first lactation and second gestation overlapped, the time from conception to the date of drying off was used. The mean length was 150 days. The average length of time from first service in the second reproduction cycle to conception was 51 days. This ranged from 0 days for cows conceiving at first service to an extreme of 302 days for one cow bred repeatedly until she conceived. There was no significant correlation between this measure of fertility and FCM yield or inbreeding of the cow in this sample. However, only cows that had a normal second gestation period are included. Assuming a 21-day estrous cycle, the 51-day mean period would indicate about 2.4 services per conception for these 171 cows.

The regression of FCM yield on inbreeding as shown in Table 5 indicates a highly significant reduction in yield of some 50 lb. FCM per unit increase in inbreeding. Likewise, the reduction of nearly 3 lb. in body weight for each per cent increase in inbreeding is highly significant.

TABLE 5

Regression coefficients of FCM production, body weight, and gestation length on inbreeding, and of gestation length on FCM production

Variables measured		Regression of Y on X
Y	X	
FCM *(lb.)*	F of cow *(%)*	-49.7 ± 14.9 lb.**
Weight of cow *(lb.)*	F of cow *(%)*	-2.9 ± 0.5 lb.**
Gest. length *(days)*	F of cow *(%)*	$+0.06 \pm 0.030$ days*
Gest. length *(days)*	FCM (100 lb.)	$+0.03 \pm 0.015$ days*

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 6

Correlation and partial correlation coefficients involving gestation length, performance traits, and inbreeding of cow

Variables			
Y	X	r_{xy}	$r_{xy \cdot F}$[a]
Gest. length	FCM yield	+0.15*	+0.19*
Gest. length	Weight of cow	−0.15*	−0.11
Gest. length	Weight gain	−0.01	−0.01
Gest. length	Service to conception	+0.07	+0.06
Gest. length	Overlap gest.-lact.	−0.06	−0.06
FCM yield	Overlap gest.-lact.	+0.01	+0.01
FCM yield	Service to conception	+0.10	+0.12
FCM yield	Weight gain	−0.17*	−0.18*

* Significant at the 5% level.

[a] $r_{xy \cdot F}$ = partial correlation with inbreeding of cow held constant.

Correlation coefficients between the several variables considered and the partial coefficients where inbreeding effects are held constant are give in Table 6.

DISCUSSION

Year and season. Analyses of variance indicate that neither yearly nor seasonal differences affected the variation in gestation length. However, a statistically significant year × season interaction suggests the existence of short-term time trends not associated with season. Such short-term trends may reflect the effect of uncontrolled management procedures that operated on the cows' pregnancy durations through complex mechanisms of the sort to be discussed in the section dealing with interrelationships between gestation length and performance.

Brakel *et al. (2)* in their extensive bibliography on gestation length include several studies that deal with the effect of season on gestation length in the bovine. In general, the results are negative or indicate some slight effect usually not at a level of statistical significance. In concert, the results do not fit into a marked pattern such as Howell and Rollins *(12)* report in a study of the horse. Brakel *et al. (2)*, in their own study, report a highly significant difference (2.07 days) between spring and autumn calvings. Jafar *et al. (14)*, in their study of Holsteins, found no effect of yearly or seasonal variation on gestation length.

In the bovine, the so-called seasonal effect is apparently confounded with the effect of other variables not integrally related to season. For the horse, Howell and Rollins *(12)* present evidence showing seasonal effect on gestation length to be independent of the mare's level of nutrition.

Inbreeding. The effect of inbreeding of the dam on gestation length was analyzed by estimating partial regression coefficients within groups of dams mated to 22 different sires, with inbreeding of the calf held constant. This set of partial regression coefficients was statistically significant at the 1% level of probability. However, they were not homogeneous. That is to say, the hypothesis that they were all estimates of the same parameter was rejected at the 1% level of probability. A possible explanation of this lack of homogeneity is that some other variable is involved in a cause-and-effect network with

inbreeding of dam and gestation length. Later in the discussion of interrelationships between gestation length and performance, evidence is presented regarding a complex of this type involving lactation of the dam. There it is shown that inbreeding of dam is negatively correlated with lactation; that lactation is positively correlated with gestation length; and that gestation length is positively correlated with inbreeding of dam.

The effect of inbreeding of the calf on gestation length was found to be not statistically significant. However, the relation of variation in birth weight to this effect merits investigation. Several studies have shown birth weight to be negatively correlated with inbreeding of calf and positively correlated with gestation length *(2, 5, 28, 39)*.

With these data an intra-sire intra-year covariance analysis of inbreeding of dam, inbreeding of calf, gestation length, and birth weight was made. The records involved information on 680 calves sired by 13 bulls. The partial regression coefficients of birth weight (in pounds) on inbreeding of calf and gestation length were -0.061 ± 0.028 and 0.360 ± 0.054, respectively. Next, an analysis of the same set of data was made with gestation length as the dependent variable and inbreeding of dam, inbreeding of calf, and birth weight as the independent variables. The partial regression coefficients of gestation length on inbreeding of calf and on birth weight were -0.015 ± 0.020 and 0.181 ± 0.027, respectively. These imply that the lack of a relation between inbreeding of calf and gestation length is not due to a confounding of the effects of joint variability of gestation length, birth weight, and inbreeding of calf. Jafar *et al. (14)*, in studying Holsteins, found no statistically significant effect of inbreeding of calf or inbreeding of dam on gestation length.

Thus it appears that, although the inbred calf is smaller at birth and less viable than the non-inbred *(27)*, its inbred condition does not affect the mechanism that induces parturition.

Partitioning of variance. The phenotypic variance (after adjusting the records for variation due to sex and calving sequence differences) was partitioned into the following components expressed as percentages: $G = 30$, $G_D = 31$, $M = 0$, and $E = 39$. These symbols are defined in Table 4. Because of analytical complications arising from the relationship between inbred families, no attempt was made to estimate a component of variance associated with dominance deviations from the additive genetic scheme. Jafar *et al. (14)* in their study of Holsteins, working with records adjusted for sex and calving sequence, estimated that 48% of the phenotypic variance was due to differences in the genotypes of calves. This was broken down as follows: 32% due to differences in additive genotypes and 16% due to dominance deviations from the additive genetic scheme.

Taken at its face value, the estimate that 16% of the phenotypic variance is due to dominance deviations from the additive genetic scheme implies that inbreeding of the calf should have some effect on gestation length. Inbreeding of the calf was found to have no statistically significant effect on gestation length in either Jafar's study or the present one. In the former study, the partial

regression coefficient of gestation length on inbreeding of calf with inbreeding of dam held constant was –0.01 and in the latter it was +0.02.

In the present study, 31% of the phenotypic variance is attributed to permanent differences between dams ($G_D = 31$). These differences are of an additive genetic kind. No dam differences of a permanent non-genetic type were found ($M = 0$). Jafar *et al. (14)* estimate 6% of the phenotypic variance to be due to additive genetic differences among dams and 15% to a combination of dominance deviations and permanent environmental causes effective directly through the dam. In the present study no attempt was made to estimate the magnitude of the effect of the dam's dominance deviations from the additive genetic scheme. The existence of such dominance effects would be compatible with the statistically significant effect of inbreeding of dam on gestation length that was found.

Heritability estimates of gestation length for cattle, sheep, and horses fall in the intermediate range of about 30% to 50%. The magnitude of the heritability estimates clearly answers affirmatively the old question: Does the sire influence the length of time his offspring is carried in utero?

Table 7 presents estimates of heritability of gestation length for cattle, horses, and sheep.

TABLE 7

Estimates of heritability of gestation length in cattle, horses, and sheep

Authors	Breed	Approx. number of animals included in study	Heritability estimate (%)	Comments
Rollins *et. al.* (present study)	Jersey cattle	1,000	30	Data adjusted for sex and calving sequence differences prior to estimation of heritability.
Jafar *et. al.* (1950)	Holstein cattle	350	32	Same as above.
Düring (1937)	Swedish Friesian cattle	900	43	Calculated as 4x paternal half-sib correlation on basis of material obtained from analysis of variance tables given in the author's paper.
Brakel *et. al.* (1952)	Ayrshire cattle	100 pairs	64	Based on 2x offspring dam correlation given by the authors in their paper.
	Jersey cattle	100 pairs	40	Same as above.
Rollins *et. al.* (1951)	Arabian horse	190	36	Data adjusted for effects of season of breeding and level of nutrition of the mare prior to estimation of heritability.
Mauch (1937)	Arab, Lipizzanser, Gidran, and Nonius horses[a]	1,500	27	Data not adjusted for season effects — an important source of variation. Heritability estimate (4x paternal half-sib correlation) based on data presented in the author's paper.
Terrill *et. al.* (1947)	Rambouillet sheep	1,600	30 to 40	Data adjusted for tangible environmental effects prior to estimation of heritability.

[a] All of these breeds of light horses founded on Arab blood.

Age of dam. A review of the literature leads to the conclusion that the variation in gestation length resulting from variation in age of dam, considered as a property of aging per se, is at most relatively small compared with the variation induced by the range of environments to which cattle are exposed.

The strongest suggestion of an age-of-dam effect is that first-calf heifers carry their calves for a shorter period than do older cows. Although this result is not universally reported, it is found in a number of studies, five of which contain 1,200 to 10,000 records *(2, 11, 13, 14, 16, 22)*. Other studies, two of which contain about 1,000 records each, report that there is no difference in performance between these two age classes *(3, 15, 17, 18, 19, 38)*. None of the studies reviewed reported that first-calf heifers carried their calves longer than did older cows.

Age-of-dam effects are statistically highly significant in the present study. The mean gestation length for the first calving sequence was 1.3 days shorter than for the second. Second calves were carried 1.2 days less than third calves. There were no significant differences nor was there any trend among calving sequences 3 and up. These apparent age-of-dam effects may be largely a result of herd management. This hypothesis will be developed in the next section.

Interrelationships between gestation length and performance. Conditions of stress brought about by decreased food intake or other adverse environmental changes may lengthen gestation. Howell and Rollins *(12)* found that the gestation period of horses on a lowered plane of nutrition was lengthened 4 days. They cite the papers of two German workers who observed similar effects of lowered feed intake in horses. Kment *(20)* and Ineichen *(13)* both state that poor conditions of feeding and management or severe weather definitely lengthen the gestation period of cattle.

The cows in the present study were raised on a high plane of nutrition. They received some 7 to 8 lb. of barley per day in addition to good alfalfa hay from 12 months of age to first freshening at about 26 months. They continued to be fed heavily during their first lactation. After completing their first record, they were placed on a considerably lower and more variable plane of nutrition for the remainder of their productive lives. Thus, their first gestation proceeded while the animals were on a relatively high plane of nutrition. The second calf was carried an average of 150 days during first lactation. Most cows were shifted to a lower plane of nutrition at the end of their first lactation. Because of this management policy, it was believed that the length of time the first lactation and second gestation periods overlapped might have an effect on gestation length. However, a statistically nonsignificant correlation of –0.06 was found between gestation length and this overlap period (Table 6). The partial correlation holding FCM yield constant was also –0.06. The length of the overlap period is determined jointly by the management policy for a herd and the fertility of the cows and the sires to which they are bred. Early rebreeding after first calving, plus high fertility, lengthens this period. Since inbreeding had little effect on fertility as measured here, it likewise had little effect on the overlap period. The length of the overlap period had no effect on

FCM yield for these 305-day records. Dickerson *(6)* has shown calving interval to be of little importance in 10-month records. The correlation between milk production and the time it took to settle a cow was +0.10. Although the coefficient is statistically nonsignificant, its positive direction may be consistent with a general pattern.

The amount of milk produced in the first lactation appeared to have some effect on second gestation length. A positive regression of gestation length on FCM yield of +0.03 days per 100-lb. increase in FCM yield was found in this study. This regression coefficient was significantly different from zero at the 5% level of probability. The corresponding correlation was +0.15. When corrected for inbreeding of dam, the partial correlation between FCM and gestation length is +0.19, which is just short of the 1% level of significance. This is the first report of a significant relationship between these two variables. Alexander *(1)* noted a slight but statistically insignificant positive relationship between duration of gestation and FCM yield. Several studies have reported no apparent relationship between gestation length and milk production in the cow *(3, 23, 29, 34, 36)*. Terrill and Hazel *(34)* noted the gestation periods of ewes in good milk to average 0.45 day longer than those of ewes classed as poor yielders.

The relations observed in this study between milk production and the time taken to settle a cow and between milk production and gestation length suggest the hypothesis that continued selection for high milk yield can result in the production of cows with unbalanced genotypes in respect to fitness. Such cows tend to produce milk at such a rate as to induce internal stress conditions that manifest themselves in the above-mentioned aspects of lower reproductive fitness. This phenomenon falls in the province of genetic homeostasis discussed by Lerner *(24)*.

In the present study the variation in gestation length associated with differences in age of dam may largely reflect the results of management. As previously described, bred heifers and first-calf cows were on a more adequate nutritive regime than were older cows and hence may have been subject to less physiological stress during or just before gestation than the older cows. In this case the effect on gestation length of variation in conditions external to the cow are under consideration rather than the effect on gestation length of variation between genotypes selected for milk yield. In each situation, however, there is the common element, viz., the lowering of reproductive fitness through a lengthening of the reproductive cycle.

In the literature there is no consistent age-of-dam effect except that first-calf heifers carry their calves a shorter time than do older cows. In this study there is a marked change in gestation length with each management change associated with age. These management changes divided the cows into three groups: bred heifers, first-calf cows, and older cows. There were no significant differences nor was there any trend among gestation lengths of cows of different ages in the group of older cows. Hence, it seems reasonable to assume that the apparent

age-of-dam effect on gestation length in this study is largely a reflection of management practices.

A hormonal basis for the effect of heavy lactation on gestation has been suggested by Turpeinen *(35)*. He demonstrated that in the mouse prolongation of gestation was proportional to the number of young suckled. The injection of progesterone or lactogenic hormone causes a prolonged gestation in mice and rats. Thus, it appears that if in the cow FCM yield has a positive correlation with gestation length, the stress mechanism may operate through the hormonal complex associated with high production. Searle *(30)* reports a high correlation between the number suckled in mouse litters and the length of gestation. He noted that gestation may extend to 5 weeks in lactating dams as compared with the expected 3 weeks in nonlactating dams.

A negative correlation of –0.15 was found between gestation length and body weight (Table 6). However, the partial correlation with inbreeding of the cow held constant was –0.11, a figure not significant at the 5% level. Knapp *et al. (21)* report a correlation of +0.001 between length of gestation and weight of dam in beef Shorthorns. Vukavic *(36)* likewise found no correlation betwen the length of the gestation period and live weight of Simmental cows. No significant correlation was indicated between gestation length and a cow's weight gain during her first lactation (Table 6). The statistically significant negative correlation of –0.17 between FCM yield and weight change indicated that the higher producers tended to gain less body weight during their first lactation. These animals are growing during their first lactation. The negative correlation, an indication of reduced growth rate of high producers, is another sign of interference with the well-being of the animal.

SUMMARY AND CONCLUSIONS

The records of 1,353 gestation periods of 427 cows of the University of California inbred Jersey herd were used in this study. Inbreeding ranged from 0 to 62% and averaged 11% for cows and 19% for calves.

The mean length of the uncorrected gestation periods was 282.7 days and the standard deviation was 5.4 days. Sex and calving sequence were important sources of variation. Male calves were carried in utero 2 days longer than female calves. First calves were carried 1.3 days less than second calves, and second calves 1.2 days less than third calves. From the third calving sequence on, no significant differences or trend was found. Neither year nor season had significant effects on gestation length. The significant year × season interaction was interpreted to indicate short-term time trends not associated with season of year.

A highly significant regression of birth weight on gestation length of 0.36 lb. per day was found.

There was no evidence that inbreeding of the calf affected the length of time it was carried in utero. The inbreeding of the dam was positively correlated with gestation length, but there was evidence that this relationship might be confounded with other variable factors, including, possibly, milk production.

A partitioning of the phenotypic variance yielded the following estimates of the relative importance of the sources of variability: additive genetic differences between calves, 30%; additive genetic differences between dams, 31%; permanent differences between dams other than those included above, 0%; unanalyzed residual effects, 39%.

Positive correlations were found between milk production and the time to settle a cow and between milk production and gestation length. The latter correlation is statistically significant at the 5% level of probability, whereas the former is not. An hypothesis is discussed in which the relationships between the above variables are interpreted in terms of genetic homeostasis.

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A STUDY OF VARIATIONS IN SECRETION OF Ca^{45} BY THE MAMMARY GLANDS OF DAIRY COWS[1,2]

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A better understanding of the causes of low blood calcium after parturition is important for proper control of milk fever. This series of experiments has helped clarify some of the questions regarding calcium metabolism, though no satisfactory explanation for the excessive proportion of radioactive calcium in milk has been found. *Editor.*

The calcium metabolism of the lactating dairy cow proceeds at a high rate because of the secretion into milk of 20 to 30 g. of calcium each day. This is about four times the calculated fecal metabolic loss of calcium by the cow *(16)*. Balance studies have shown that the lactating cow is normally in negative calcium balance in the major part of the lactation period, even when adequately fed *(3)*. The functioning udder has been shown to be the cause of drastic changes in calcium content of the blood at the initiation of lactation *(12)*, and dairy cows are uniquely afflicted with an ailment, milk fever, caused by low blood calcium after parturition *(8)*. An understanding of the secretion of milk calcium and its probable precursor forms and sources has been sought in previous studies by the use of radioactive calcium by which blood calcium and feed calcium have been labeled *(15)*. These studies indicated that some cows apparently were able to concentrate radioactive calcium in their milk after oral dosing to a greater extent than they concentrated stable calcium, although this phenomenon was not so noticeable after intravenous dosing. After both oral and intravenous doses, the proportion of radioactive to stable calcium remained higher in milk than in blood, even after correction for time of milk secretion. Since this difference in the peak specific activity of blood and milk calcium was

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at variance with the expected precursor-product relationship *(18)*, the additional studies presented in this article were conducted to seek a further explanation for this phenomenon.

MATERIALS AND METHODS

The cows used in this study either were all Jerseys or carried at least 50% Jersey blood and were mature in age. They varied widely in milking ability and stage of lactation through the series of experiments. They were fed a medium to poor quality mixed hay and commercial 16% protein dairy feed which was fortified with mineral supplements containing adequate calcium, phosphorus, and minor elements. Dosing, sampling, and analyses were done by the methods previously described *(2, 15)*. Intravenous injection of 10 units oxytocin was given prior to all milkings during the first 2 days of each experiment and for all later periods in which frequent milkings were made. Special methods for individual experiments are described below with the discussion of those experiments. Specific activity of calcium has been calculated as the percentage of the dose of Ca^{45} times 10^4 per milligram of total calcium. In plotting the specific activities of calcium from milk and urine, the median time between sample collections has been used rather than the time of collection. This correction for time of secretion of milk was assumed to be adequate because it was found that the specific activities of first and last drawn milk after oxytocin-induced milk ejection at one milking period differed only slightly, being 1.93 and 1.83, respectively. All changes in specific activity with time have been plotted on semilogarithmic scales, according to time after dosing.

RESULTS

Comparing oral and intravenous doses. Since comparisons of specific activity of blood and milk calcium after oral and intravenous doses in the same cow had not been presented previously, these data were obtained from two cows in mid-lactation. They were given oral doses of approximately 5 mc. $Ca^{45}Cl_2$ December 14, 1951, and February 1, 1952. They were dosed intravenously with 1.75 mc. of $Ca^{45}Cl_2$ March 5, 1952. The results of these trials with cow 1 are shown in Figures 1 to 3. Very similar results were obtained in the trials with cow 5.

These figures show that the declining curve of blood calcium specific activity after an intravenous dose passed through the peak of the time-corrected milk curve, but later a slight delay in calcium secretion into milk was indicated. In the straight declining phase of the curves the blood and milk values were parallel but separated by a time factor of about 8 hours. These differences might be explained by a calcium reservoir in the udder requiring an average of 8 hours for milk precursor calcium to traverse. The specific activity curves after oral doses were somewhat different. In the December trials the peaks of milk and blood calcium specific activity were nearly equal, but in the February trials milk reached a higher specific activity than blood, as had been noted

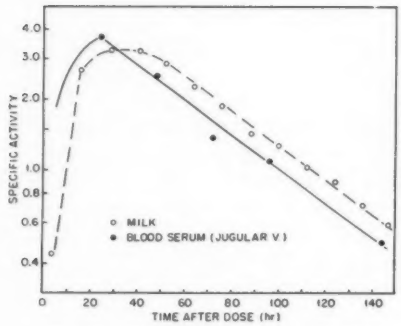

FIG. 1. Comparison of the specific activity of blood and milk calcium after an oral dose of Ca^{45} given to cow 1 December 14, 1951.

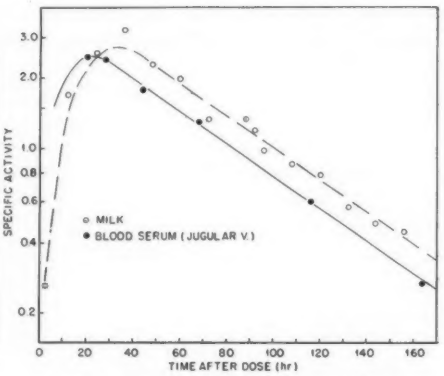

FIG. 2. Comparison of the specific activity of blood and milk calcium after an oral dose of Ca^{45} given to cow 1 February 1, 1952.

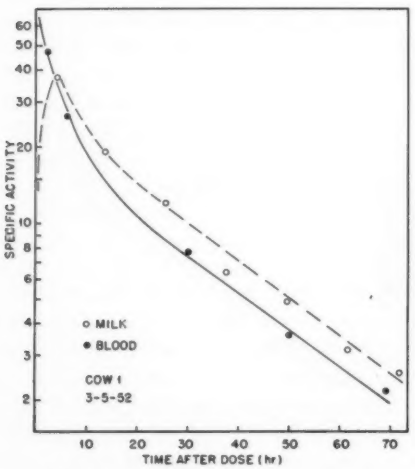

FIG. 3. Comparison of the specific activity of blood and milk calcium after an intravenous dose of Ca^{45} given to cow 1 March 5, 1952.

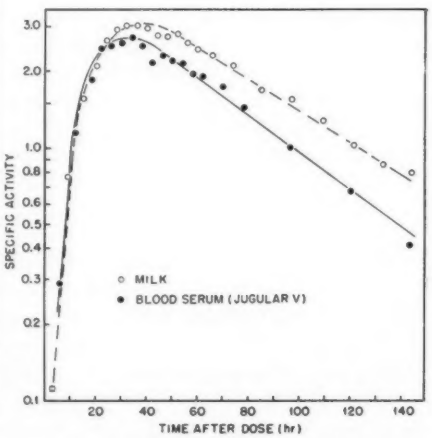

FIG. 4. Comparison of the specific activity of blood and milk calcium after an oral dose of Ca^{45} given to cow 5 with collections of samples at 4 to 6 hour intervals.

before. The straight declining blood and milk curves for cow 1 were nearly parallel, but for cow 5 they seemed to diverge in later samplings. The time differences between the curves for cow 1 were 13 and 17 hours and those for cow 5 were 14 to 20 hours and 20 to 26 hours, respectively, in the two trials. If the udder were preferentially concentrating radioactive calcium, which after an oral dose is a representative tracer of feed calcium, the specific activity decline curves of blood and milk would be expected to diverge. The observation

that they diverged with one cow and not with the other, irrespective of the relation of milk and blood calcium specific activities at the peaks, makes the interpretation of this phenomenon difficult. These types of inconsistencies have appeared at other times in these studies. There was a strong suggestion, however, that the cow's mammary gland may handle orally derived calcium differently from that placed into the blood stream all at one time.

Blood and milk calcium specific activity changes after oral doses. The possibility of the blood calcium reaching a higher peak specific activity than that of milk after an oral dose, but maintaining it for so short a time that it was missed in the sampling schedule, was investigated with two cows from which very frequent samples were taken in the early phase of the trial. Each cow was dosed orally with about 5 mc. of $Ca^{45}Cl_2$. If the contents of the dosing capsule were not thoroughly mixed in the cow's stomach but were rapidly shunted to an area where the major portion was absorbed, such a transitory peak blood specific activity would be possible.

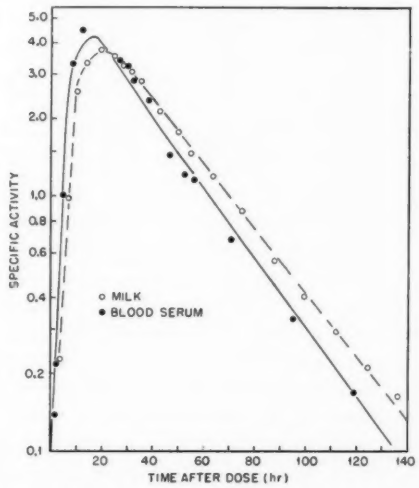

FIG. 5. Comparison of the specific gravity of blood and milk calcium after an oral dose of Ca^{45} given to cow 11 with frequent collection of samples.

The results of these trials are shown in Figures 4 and 5. Even with samples taken at 4-hour intervals, the specific activity of none of the blood samples from cow 5 was as high as that of the seven highest milk samples (Figure 4). The peak specific activity of the blood was not attained until 34 hours after dosing, which indicates a rather slow absorption of the dose. The peak specific activity appeared in the milk at about the same time as the blood peak. In cow 11, however, peak specific activity was found in the 12-hour blood samples, which exceeded the highest specific activity from any of the milk samples (Figure 5). The peak for specific activity in milk occurred at about 20 hours. These

two cows clearly absorbed and metabolized their ingested calcium at different rates. Cow 11, which absorbed the dose more rapidly, failed to concentrate the radioactive calcium in the milk as did cow 5, which absorbed the dose more slowly. The specific activity curves of the blood and milk of both cows appeared to diverge slightly in the declining or presumably post-absorptive phase. These first experiments seemed to confirm the previous observation *(15)* that the mammary gland was concentrating radioactive calcium in preference to stable calcium after an oral dose in some cows at some periods; but the effect was not always observed.

Test for a high specific activity blood fraction by electrophoresis after adding Ca^{45}. Although Visek *et al. (15)* had not been able to find blood fractions that differed significantly in specific activity from the whole blood calcium by means of ultrafiltration and adsorption, the possibility that serum calcium is not all of equal mobility still exists. One example which might be due to differences in mobility of radioactive calcium in the blood is the observation that specific activity of blood calcium of the new-born calf from a cow dosed before parturition was 2.5 times that of the cow *(15)*. A further attempt to detect differences in blood calcium fractions was made by a combination of paper electrophoresis and autoradiographic techniques. The method of Gordon *et al. (4)* was used. All strips were moistened with 0.05 *M* veronal buffer (pH 8.5) and 5 to 10 λ of sample were applied to the moist strip before a potential gradient of 5 volts per centimeter was established.

The results of three typical tests are shown in Figure 6. Strip *a* is an autoradiogram of a sample of $Ca^{45}Cl_2$ after 1 hour electrophoresis in veronal containing 0.9% NaCl. All of the radioactive calcium had migrated a distance of 7 cm. Strips *b* and *c* resulted from the same treatment of bovine serum, which had been incubated with Ca^{45}. The autoradiogram is superimposed upon the paper strip, which was stained with bromphenol blue to show the proteins. It is evident that the radiocalcium had migrated the same distance as the calcium chloride (strip *a*) and that it had separated cleanly from the plasma proteins except for a few intensely radioactive spots at the site of sample application (strip *b*). These spots were found to be the result of precipitation of calcium salts in blood plasma by the addition of relatively low specific activity Ca^{45}. When radiocalcium of higher specific activity was added to serum (strip *c*) all the radioactivity was found in the calcium-ion band. These results show that the bond between calcium and serum protein is so weak that it is easily and rapidly broken by the electrophoretic removal of free calcium ions from the medium surrounding the protein molecules.

To compare the physico-chemical state of calcium in milk to that in blood, ultrafiltration and paper electrophoresis were performed on a milk sample incubated with $Ca^{45}Cl_2$ for several hours. The electrophoretic pattern lacked definition. Radioactivity was distributed more or less evenly between the origin and an ionic-calcium band on the cathode side, and radioactivity was also found with the protein bands toward the anode. Such a picture could be explained by the presence of protein-bound or other colloidal calcium, which decomposed

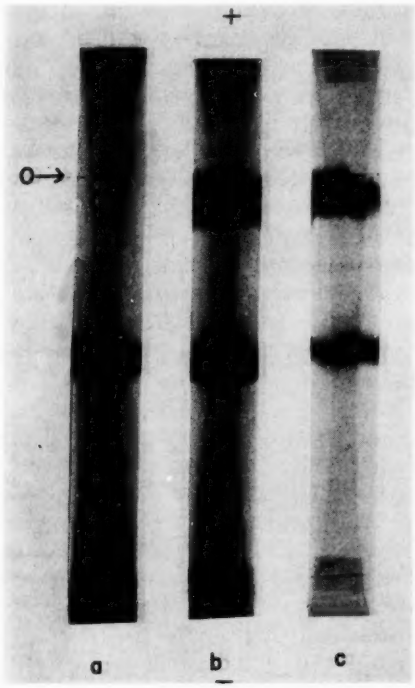

FIG. 6. Autoradiograms of $Ca^{45}Cl_2$ (a) and $Ca^{45}Cl_2$ in blood serum (b and c) superimposed on paper electrophoretic strips stained to show the serum proteins near 0. The lower bands are the images of Ca^{45}. Sample was applied at 0→.

slowly under the influence of electrophoretic withdrawal of Ca ions from the medium. Ultrafiltration of the same milk sample over a period of 16 hours in the refrigerator indicated the presence of 72% nondiffusible calcium, a value closely agreeing with the results of Magee and Harvey *(10)*. Ca^{45} determinations on the ultrafiltrate showed that its specific activity exceeded the specific activity of whole milk by 33%. Apparently the calcium in milk did not exchange freely with ionic calcium as it had with serum calcium.

Effect on blood and milk calcium of intravenous injection of large amounts of stable calcium. Although all methods of blood fractionation used had failed to show a high specific activity component, the appearance of the excess milk calcium specific activity after oral doses suggested that recently absorbed calcium might be used for milk formation in slight preference over the total blood calcium. If this were true, it was reasoned that adding extra calcium to the blood to simulate newly absorbed calcium should produce a greater effect upon the specific activity of milk calcium than upon the blood of a cow in the straight declining phase after an oral dose. To test this hypothesis, a cow that had previously shown the apparent Ca^{45} concentrating effect in milk was used in

three different trials. In each trial, the cow was given a 5-mc. oral dose of $Ca^{45}Cl_2$, and blood and milk samples were collected as previously described. Beginning at about 30 hours after dosing, injections of solutions furnishing 0.5 g. of Ca were made by intravenous catheter at 30-minute intervals for 8 hours. During this injection schedule, milkings were made at 3- to 4-hour intervals. Blood samples were taken just before an injection at 2-hour intervals or oftener. In the first trial, $CaCl_2$ solution was used. In the second trial, $CaCl_2$ and NaH_2PO_4 solutions were used in proper proportion to maintain a normal Ca : P ratio in the blood. Calcium lactate was used in the third trial.

The results of the preliminary oral dose with cow 8 and the injection of calcium plus phosphorus trial with the same cow are shown in Figures 7 and 8.

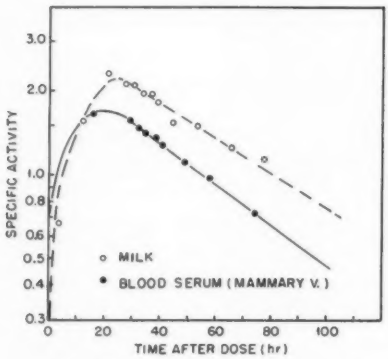

FIG. 7. Comparison of the specific activity of blood and milk calcium of cow 8 after an oral dose of Ca^{45} given near the peak of her lactation, December 10, 1952.

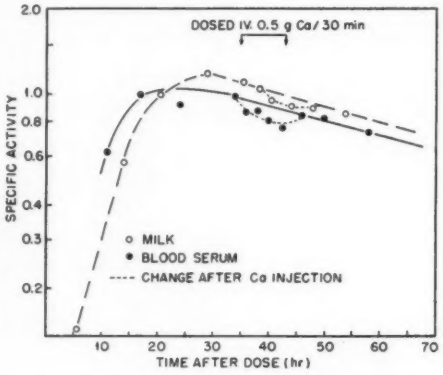

FIG. 8. Changes in the specific activity of blood and milk calcium after an oral dose of Ca^{45} given to cow 8 March 4, 1953, followed by 8 hours of injecting $CaCl_2$ and NaH_2PO_4 March 5.

It is shown in these graphs that a much greater difference between milk and blood calcium specific activity was found in the preliminary trial than in the later ones. In the former trial the cow was at the peak of her lactation, 5 weeks, in December, 1952. The $CaCl_2$ trial was made in January, 1953, and the next trial in March, 1953. In all trials the specific activity of calcium from blood and milk declined at the same rate, producing parallel curves. The results with $CaCl_2$ or calcium lactate were very similar to those with calcium and phosphorus.

Contrary to the above hypothesis, the specific activity of milk was influenced less by massive injections of stable calcium than was that of blood. The plotted differences are probably less than the true average differences because the blood was sampled at its highest specific activity within each 30-minute injection period. The $CaCl_2$ injections decreased blood calcium specific activity 19%, but at the same time milk calcium specific activity decreased only 11%. Calcium and phosphorus injected together caused a 12% decrease in blood and

an 8% decrease in milk calcium specific activity. In neither trial did the altered specific activity of milk calcium decline to the normal expected blood level. Although the distance between the milk and blood specific activity curves indicated a probable average lag of 7 and 9 hours, respectively, in the two trials, the lag in appearance of the effects of the calcium injections on milk calcium specific activity was less than 4 hours. This indicates that part of the milk calcium is derived rapidly from the blood while another part may pass through an udder reservoir, which would cause a delay in the movement from blood to milk. The contribution of this udder calcium reservoir to the milk explains why the specific activity of milk calcium was affected less than blood calcium by the 8 hours of calcium injections. These results indicate that injected calcium is not preferentially used for milk formation to the extent that it may explain the peak differences in blood and milk calcium specific activities.

Isotopic analysis of blood and milk calcium. If the cow's mammary gland were capable of preferentially selecting Ca^{45} from the blood for secretion into milk, the differences in milk and blood specific activities might be due simply to such an isotope effect. If this were true, milk should also be richer than blood in stable calcium isotopes Ca^{44} and Ca^{46}. To determine this, blood and milk samples were secured from a cow in early lactation. Calcium was collected from each as the oxalate precipitate and then converted to the nitrate and analyzed by the mass spectrograph. The percentages of six stable calcium isotopes found in blood and milk samples are shown in Table 1. Since the

TABLE 1

The concentration of six stable calcium isotopes in blood and milk drawn from the same cow

Calcium isotope	Concentration in atom per cent	
	Blood	Milk
(Mass No.)	*(%)*	*(%)*
40	96.81	96.81
42	0.68	0.66
43	0.14	0.15
44	2.18	2.19
46	0.003	0.003
48	0.189	0.193

values of these six isotopes were nearly identical from the two sources of calcium, it seems unlikely that the mammary gland of the cow concentrates Ca^{45}, per se; thus, the higher specific activity of milk may not be explained by an isotope effect.

Comparison of specific activity changes of calcium in arterial and venous blood, urine, and milk of cows after an oral dose of Ca^{45}. In all of the previous trials blood samples had been obtained from the jugular vein. Since blood calcium is constantly being exchanged with bone calcium, the blood is constantly being depleted of recently absorbed calcium (in this instance Ca^{45}) which is replaced by calcium (mostly stable Ca) from the skeletal and tissue stores *(7, 14)*. Theoretically, this could result in venous calcium having a very slightly

lower specific activity than arterial calcium. Another possible change in blood calcium could occur as the result of the removal of calcium by the udder. An attempt was made to detect such changes in this experiment.

Three cows were used in these trials. About a week before dosing, the carotid artery of each cow was displaced so that it lay just under a fold of the skin of the neck. Arterial blood samples were then easily obtained by hypodermic needle and syringe. Each cow was dosed with 5 mc. of $Ca^{45}Cl_2$ orally. Blood samples were taken at frequent intervals simultaneously from the carotid artery, jugular vein, and subcutaneous abdominal vein, which was presumed to contain mostly blood from the mammary gland. Milk and urine samples were obtained twice daily. Because of its very low calcium content, urine was wet-ashed in glass beakers to insure reliable specific activity values.

The specific activity values of calcium from each source from cows 7 and 9 are presented in Figures 9 and 10. Similar results were secured from cow 8.

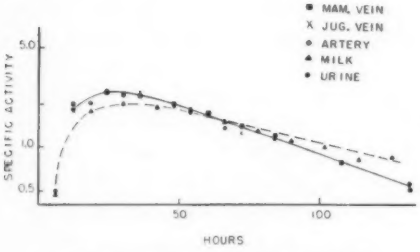

FIG. 9. Comparisons of the specific activity of calcium from milk (broken line) and urine and blood from the carotid artery, jugular vein, and subcutaneous abdominal vein (solid line) of cow 7 after an oral dose of Ca^{45}.

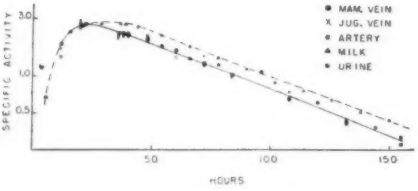

FIG. 10. Comparisons of the specific activity of calcium from milk (broken line) and urine and blood from the carotid artery, jugular vein, and subcutaneous abdominal vein (solid line) of cow 9 after an oral dose of Ca^{45}.

No consistent difference in specific activities of calcium from urine, arterial blood, jugular vein blood, or subcutaneous abdominal (mammary) vein blood was found. Therefore, the trend of these values has been represented by one line. The specific activity of milk calcium from these cows did not reach a higher level than that of blood except in the declining phase of the curves, at which time the specific activity of calcium from the three blood sources continued to remain nearly identical. If slight differences in blood specific activity did exist, the analytical methods were not precise enough to demonstrate them. The agreement of specific activity of urine and blood calcium in nearly all samples indicates that the calcium excreted by the kidney is representative of all of the blood calcium, lending further doubt as to the existence of a high specific activity blood fraction.

Since only one of the cows (No. 9 in Figure 10) used in this experiment demonstrated the differences in milk and blood calcium specific activity expected on the basis of previous trials, these results may not have been typical of cows

in which this effect did occur. However, cows 7 and 8 had exhibited definite differences in milk and blood calcium specific activities in previous experiments. The specific activities of blood calcium from the three sources differed no more for cow 9 than for the two cows showing lesser blood-milk differences. Two other trials comparing the specific activities of serum calcium from the jugular and subcutaneous abdominal veins also failed to show differences large enough or consistent enough to explain the blood and milk differences.

Other observations on the secretion of Ca^{45} into milk. Cow 8, which had a history of milk fever in previous lactations, repeatedly showed a higher peak specific activity in milk than in blood calcium, and the differences between blood and milk calcium specific activities were wider in early lactation (Figure 7) than in later lactation (Figure 8). Another cow (No. 87) with a history of milk fever was also used in Ca^{45} secretion studies and was given oral doses in the first and fourth months of lactation. The results from these trials are shown in Figure 11. As with cow 8, wide differences in milk and blood calcium

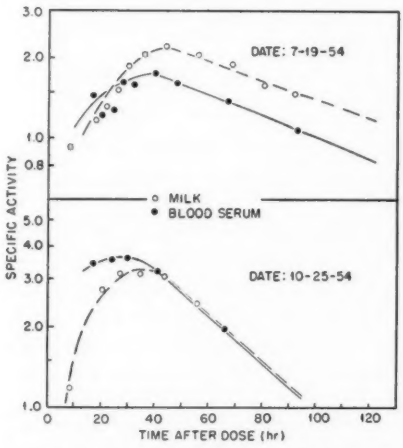

FIG. 11. Comparisons of the specific activity of blood and milk calcium of cow 87 after oral doses of Ca^{45} July 19, 1954 (one month after calving with milk fever) and October 25, after milk yield had declined to a low level.

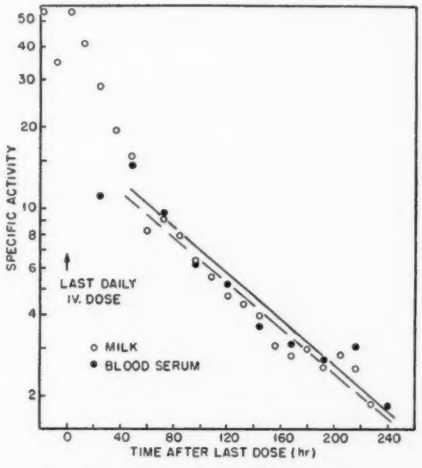

FIG. 12. Comparison of the specific activity of blood and milk calcium from cow 6 for 10 days after the last of 10 daily intravenous injections of Ca^{45}, July, 1952.

specific activities were observed at the peak of lactation, and practically no differences were found at the later trial. These results suggest that the process by which the specific activities differences are produced is most active in early lactation. That the stage of lactation is just one of the factors involved, however, is shown by the results with cows 1 and 5 at two different periods (Figures 1 and 2) and by the results with cow 9 (Figure 10) and cow 11 (Figure 5), which were both good producers used in the plotted experiments at an early

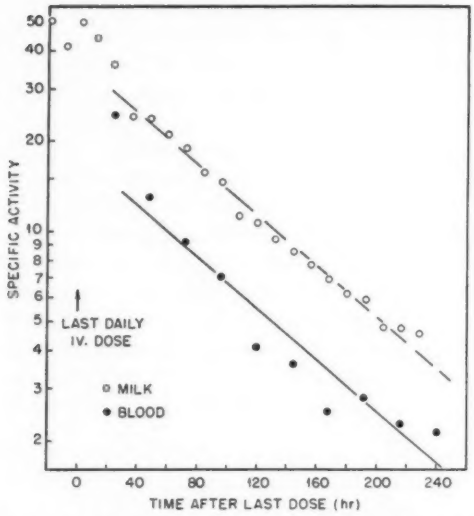

FIG. 13. Comparison of the specific activity of blood and milk calcium from cow 7 for 10 days after the last of 10 daily intravenous injections of Ca^{45}, July, 1952.

stage of lactation. In these four cows only slight or no differences in peak specific activities of blood and milk calcium were observed from experiments in the early part of lactation.

An interesting comparison of the specific activities of blood and milk calcium was made with two cows that were used in an experiment to determine endogenous fecal calcium *(16)*. These cows were given daily intravenous injections of 150 and 200 μc. of $Ca^{45}Cl_2$ for 10 days respectively, in each of two separate trials. The specific activities of milk and blood calcium for 10 days after the last injection in one trial are shown in Figures 12 and 13. The values for blood and milk for cow 6 were nearly identical in both trials. For cow 7, however, the curves for blood and milk although parallel were separated by about 72 hours in time in both trials. These results indicate again that the decline in specific activity of blood and milk calcium is generally at the same rate; therefore, the udder probably does not concentrate Ca^{45} continuously. The consistent difference in the response of the two cows indicates again the wide variations that may exist in the manner in which calcium is handled by the cow or the udder. The milk production of cow 6 in both trials was 8 to 10 lb. daily, whereas cow 7 was producing 15 to 18 lb. daily, and this production difference may have had an influence on the calcium metabolism.

DISCUSSION

This series of experiments has helped to clarify some of the questions regarding calcium metabolism by the milking cow, but the explanation for others is still obscure. No entirely satisfactory explanation of the higher specific ac-

tivity of milk calcium than blood calcium at the peak of the curves after an oral dose has appeared. This phenomenon has also been reported for phosphorus after an oral dose of P^{32} *(13)* with the supposition that the mammary gland removed from the blood a high specific activity fraction of phosphorus for milk secretion. All attempts, including those reported here and work by Visek *et al. (15)*, to demonstrate such a high specific activity fraction of calcium have resulted in negative observations. If such a blood fraction exists it must be handled differently in different cows, since not all cows have consistently shown the higher peak milk specific activity. This is not a remote possibility, however, since cows are known to differ considerably in calcium metabolism and blood calcium components just after parturition, resulting in differences in milk fever development; and, furthermore, cows in these experiments known to be susceptible to milk fever were among the most definite demonstrators of the differences in blood and milk calcium specific activity.

It is postulated that if such a high specific activity blood calcium fraction does exist, its duration is relatively short after oral dosing. Otherwise, as time progressed the specific activities of blood and milk calcium would diverge markedly rather than remain parallel, as they have in nearly all of these studies. Additional evidence against a high specific activity fraction of blood calcium is the observation that calcium in the urine and that in blood were nearly identical in specific activity. These results confirm similar observations in dogs *(5)* and humans *(1)*. If blood calcium fractions of differing specific activities were circulating, it would be unlikely that the kidney would excrete them in the same proportion as that found in blood. Furthermore, lack of specific activity differences between arterial blood calcium and mammary vein blood calcium did not indicate that the mammary gland was removing a high specific activity fraction. Since the total calcium arterial-venous differences in this study were only about 2 to 4% and the peak specific activity differences when exhibited were usually of the order of 10 to 15%, the precision of the analytical methods may not have been good enough to show such an effect. Failure to alter milk calcium specific activity as much as that of blood calcium by repeated intravenous doses of calcium suggested that blood calcium recently absorbed largely as ionic calcium may not be preferentially used for milk formation, supposing that absorbed and injected calcium would react similarly.

The mass spectrographic analyses of blood and milk calcium from one cow gave no indication of the possibility of a preferential secretion by the mammary gland of heavier than average calcium isotopes. In an experiment subsequent to the samplings for spectrographic analysis, it was found that this cow (No. 10) did not appear to concentrate Ca^{45} in her milk after an oral dose. Until the isotopic analyses can be made on blood and milk of a cow showing the concentrating effect, the possibility of isotope separation in some cows should not be completely rejected. If there were an isotope separation, however, it would not be expected that the heavy isotopes would enter into the reactions leading to milk formation at a greater rate than the light isotopes. Therefore, this explanation is given very minor consideration.

After the peaks of activity have passed, the specific activity of milk calcium corrected to the average time of secretion of the sample has in a great majority of the experiments remained above the specific activity of blood calcium as long as reliable samples could be collected. The rate of decline of both blood and milk values has been exponential, and the values have been parallel. This situation is consistent with the hypothesis that milk calcium is derived from a homogeneous portion of blood calcium but that a constant delay in the appearance of the milk calcium occurs. This delay can be explained by the existence in the udder of a calcium reservoir from which a large part of the milk calcium is eventually drawn. Such a calcium pool has been suggested by Gowen and Tobey *(6)* on the basis of excessive ash content of the udder. Monroe *(11)* found that four udders from cows in late lactation contained two to four times as much calcium as could be accounted for by the residual milk they contained. It is possible that udders from cows at the peak of lactation would show even greater stores of excess calcium. Since the total residual milk may easily equal 20% of the amount normally secured at a milking, it follows that the recently milked udder may contain enough calcium to form the amount of milk to be secreted in the next 12 hours.

Another important factor in the movement of calcium from blood through the udder to milk is the relatively low exchangeability of milk calcium, which has been demonstrated in these experiments and others *(17)*. It is postulated that calcium which enters the secretory tissue of the udder is also bound in some manner so that it does not again return easily to the blood but is primarily released or continued in processing to form milk. Such a binding process is necessary to explain the very long periods that have been observed in some cows between equal specific activities of blood and milk calcium. One cow dosed prepartum *(15)* had shown such a lag period of about 10 days for milk secreted during the first 10 days of lactation. Several cows in the experiments reported here have shown lag periods of 18 hours or more, and one period was 72 hours. Such long delays cannot be explained by the delay of movement of preformed milk through the udder, as was proposed by Lakshmanan and Kumar *(9)*. On purely physical considerations such an explanation is not plausible because the total weight of a milked udder is not equal to more than one day's milk yield.

The variability in the time lag between equal calcium specific activities of blood and milk is probably due to a combination of many factors. The size of the udder calcium reservoir and its proportionate contribution to milk calcium is one factor. The data in Figure 8 show that part of the milk calcium is derived rapidly from the blood, probably without passing through the udder reservoir. If all of the milk calcium came directly from blood as the milk was secreted, the specific activity curves of blood and milk would be expected to coincide in the declining phase, as they have in several of the experiments presented here. If the calcium binding reservoir of the udder were very large or active, proportionately more of the milk would be derived from this source, which would result in longer lag periods. Another possibility is a variation in exchange-

ability of udder calcium. The differences observed could be explained without assuming major changes in the amount of calcium reserve in the udder if exchange rates varied widely. Extremely rapid exchange would result in a minimum time lag, whereas a low exchange rate with a given size reservoir would produce a longer period between equality of blood and milk calcium specific activity. More definitive experiments upon secreting mammary tissue are necessary to clarify these questions.

SUMMARY

Experiments have been discussed in which the specific activities of milk calcium and blood calcium have been compared after intravenous and oral doses of radioactive calcium administered to lactating dairy cows, along with investigations to determine the cause of the differences observed. After intravenous doses the specific activity curve of blood calcium as it declined passed through the peak of the milk calcium curve corrected to average time of secretion. After the peak, the milk specific activity usually remained higher than the blood but continued to decline at the same rate as blood. After oral doses wide variations in response have been observed. In some animals the specific activity of milk calcium has exceeded the highest observed specific activity of blood, but in others they were nearly equal at their maximum. After the peak the decline curves have been very similar to those after an intravenous dose.

Cows that had previously developed milk fever were found to exhibit greater differences in specific activities of blood and milk calcium in early lactation than other cows; yet these differences were observed also in cows that were not highly susceptible to milk fever. No satisfactory explanation for the excessive proportion of radioactive calcium in milk has been discovered. Paper electrophoresis, arterial-venous differences, and experiments involving the constant injection of stable calcium failed to support the hypothesis that a high specific activity fraction of blood calcium existed which might be used preferentially for milk formation. Mass spectrographic analysis of blood and milk calcium of one cow showed no differences in proportions of six calcium isotopes, which indicated that the udder was not concentrating Ca^{45} per se. The existence in the udder of a variable calcium reservoir from which the calcium is not readily exchanged with blood calcium, and which is used for milk formation, has been postulated to explain the differences in time between equal specific activity values of blood and milk calcium.

ACKNOWLEDGMENTS

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TECHNICAL NOTES

SOME CARBONYL COMPOUNDS IN RAW MILK[1]

The presence of alpha-ketoglutaric acid in pasteurized milk has been reported recently by Patton and Potter *(3)*. It appears timely, therefore, to report the results of similar experiments completed at the time of publication of Patton and Potter's observations. The results confirm and supplement their findings.

A survey project has been in progress to determine the kind and concentration of various carbonyl compounds in fresh milk. During November and December, 1955, milk was obtained from 20 individual cows of the University herd. All cows were healthy, showing no clinical indication of either ketosis or mastitis. Fifty-ml. samples were obtained at the time of milking and were cooled to 4° C. The samples were immediately deproteinized by adding 5 ml. of 10% sodium tungstate, agitating for a few minutes, and then acidifying to pH 4 with 0.67 *N* sulfuric acid. The solid material was removed by centrifugation. The supernatant was filtered by suction through a Whatman No. 1 filter paper, dampened and dusted with washed silicic acid. The sediment layer was added to the filter and washed several times with 20-30 ml. of distilled water at 25° C. The filtrate was then reacted with 2,4-dinitrophenylhydrazine for 1 hour at 25° C. The phenylhydrazine derivatives were ether extracted and chromatographed for acidic components by the method of El Hawary and Thompson *(1)*. The alpha-keto acids were identified by a combination of Rf values and light absorption in the visible range. Beta-keto acids were identified by a combination of Rf values of the acid and identification of the neutral phenylhydrazone components formed by decarboxylation of the beta-keto acid phenylhydrazones. The results of analyses are summarized in Table 1.

As shown by the data, both alpha-ketoglutaric acid and pyruvic acid were found in significant concentrations in all samples. Alpha-ketoglutaric acid was present in the highest concentration of any of the keto acids studied, with an average of about 2.5 mg. per liter. Pyruvic acid was present generally in about one-tenth the concentration of alpha-ketoglutaric acid. Acetoacetic acid was present in eleven of the 20 samples, oxalacetic acid was present in eight of the samples, and oxalsuccinic acid was present in three of the samples. Alpha-ketocaproic or isocaproic acid was present in one sample analyzed.

TABLE 1

Carbonyl compounds in 20 samples of fresh raw milk from the University herd

Compounds	Concentration of carbonyl compound[a]	
	Range	Average
	(*mg/l.*)	(*mg/l.*)
Oxalsuccinic	Trace in 3 samples	
α-Ketoglutaric	0.1-8.6 in 20 samples	2.4
Oxalacetic	Trace in 10 samples	
Pyruvic	0.02-0.2 in 20 samples	0.08
Acetoacetic	Trace in 8 samples	
Formaldehyde[b]	Trace in 4 samples	
Acetaldehyde	Trace in 12 samples	
Acetone	Trace in 13 samples	

[a] Trace refers to less than 0.01 mg/liter of milk.
[b] Identified by Rf value only.

Chromatographic analyses of the neutral hydrazones by the method of Heulin *(2)* revealed the presence of acetone and acetaldehyde in all the samples studied. In addition, formaldehyde was occasionally present. Also unidentified carbonyl compounds of carbon chain length of 5 and higher, together with unidentified aromatic carbonyl compounds were present in some samples.

Standard bacterial plate counts revealed a range of 195 to 9,300 bacteria per milliliter in the 20 samples, with an average of 2,500. There was no relationship between bacterial counts and the concentration of carbonyl compounds.

Addition of 10 mg. per liter alpha-ketoglutaric acid or 2 mg. per liter of pyruvic acid to pasteurized milk had no appreciable effect on the flavor of the product. The significance of the presence of the keto acids will require additional study.

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[1] Technical Note 6:56, Department of Dairy Technology, The Ohio State University and the Ohio Agricultural Experiment Station. O.A.E.S. Journal Article No. 63-56. 1956.

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AN INEXPENSIVE URINE COLLECTING APPARATUS FOR DAIRY COWS

The problem of separate collection of urine and feces from dairy cows has been persistent. Some of the methods employed have been cited recently by Cunningham *et al.* *(1)* and Gorski *et al.* *(2)*.

The apparatus to be described is similar in principle to other apparatuses but is deemed worthy of consideration because it is inexpensive, easy to construct, and consists of materials which are readily available.

The necessary materials are: coat hanger wire, 1/16-in. rubber sheeting, foam rubber, rubber tubing, rubber cement (Alrite), F-F Branding Cement (Nebraska Salesbook Co., Lincoln), a tire patching kit, friction tape, No. 18 copper wire, aluminum sheet metal, 3-in. gooch rubber tubing, an old inner tube, a bolt or metal rod, two kneeling pads, two small pieces of ¼-in. plywood, and a fiber strap.

The apparatus is constructed as follows. The coat hanger wire is cut into six pieces and fastened together with friction tape (Figure 1).

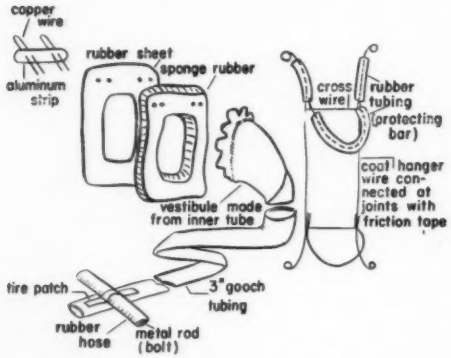

FIG. 1. Exploded-view showing component parts of the urinal.

A vestibule is fashioned from inner tubing and fastened to a rectangular piece of sheet rubber with an oval-shaped hole in it. This is accomplished by flanging the tubing and attaching the two by means of rubber cement and staples. The gooch tubing is attached to the distal end of the vestibule with rubber cement. Then a piece of foam rubber similar in shape to the sheet rubber is cemented to the outer surface of the latter. The top cross wire of the supporting frame is then attached to the whole unit with copper wire and an aluminum reinforcing strip. A metal ring is fastened around the gooch tubing one-third of the way from its distal end, and a cross piece consisting of a section of rubber hose with a metal rod in its lumen is attached to the peripheral end of the gooch tubing. The cross-piece weights the tubing and prevents it from being pulled from the urine collecting bottle which is placed to the rear of the gutter and, in our case, beneath the floor. The urinal is then completely assembled. Figure 1 diagrammatically illustrates the component parts, and Figure 2 (left side) shows the assembled urinal in cross section. The gooch tubing is omitted. Note that rubber tubing is placed around the upper arms of the wire supporting frame to prevent irritating the animal.

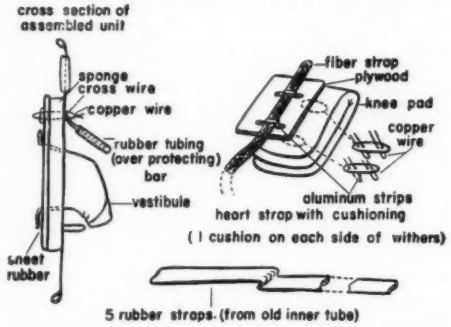

FIG. 2. Cross section of urinal and an exploded-view showing the component parts of the harness.

The critical points to be observed in constructing the apparatus are that the orifice be of sufficient size to circumscribe the opening of the vulva, and that the width of the wire supporting frame be such that it fits with ease between the pin bones. Both of these factors must be determined by observing the anatomy of each animal. The wire protruding over the vestibule is to prevent damage which might occur from the switching of the cow's tail. The wire should be covered with rubber tubing to prevent irritation to the under side of the tail.

A means of support for the urinal is satisfactorily furnished by use of a harness as illus-

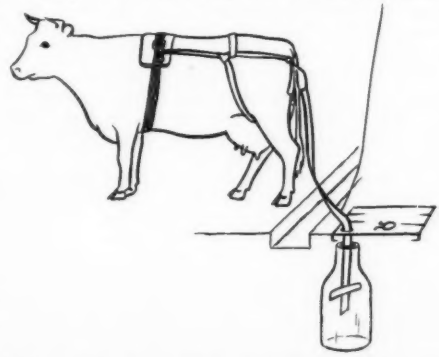

FIG. 3. The assembled apparatus in place.

trated in Figure 3. A fiber strap is cinched around the forward part of the barrel and used as the anchor point for the rest of the harness. The kneeling pads are folded (Figure 2) and placed in the region of the crops. Their function is to raise the cinch strap so that it will not cut off the circulation by pressing on the top line — thus, pressure necrosis is prevented. The plywood pieces are placed over the pads to insure that the pressure will be distributed over the entire surface area of the pads. The rest of the harness consists of five rubber straps, cut from old inner tubes, arranged to hold the urinal in place as illustrated (Figure 3).

As a stabilizing measure, branding cement is used to attach the peripheral portion of the face of the urinal in apposition with the cow. Lastly, a supporting piece of twine is attached to the ring on the gooch tubing. The harness should be checked periodically so that any damage or maladjustment may be corrected before sample contamination can occur.

Apparatuses constructed from these materials have been used for 30 digestibility trials on nine cows. The collection periods have ranged up to 11 days with little or no irritation to the animals. The uniformity of experimental results to date illustrates its effectiveness.

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AN AGAR CULTURE MEDIUM FOR LACTIC ACID STREPTOCOCCI AND LACTOBACILLI

In studies involving isolation of lactic streptococci from various sources it was observed that many of the agar media proposed for streptococci and lactobacilli did not support growth of fastidious strains of these organisms. Some media that did support growth had disadvantages from standpoint of ease of preparation or clarity of medium. Consequently a wider investigation was conducted primarily to evaluate additional agar culture media for their ability to support colony growth of fastidious lactic streptococci, particularly *Streptococcus cremoris* and *S. lactis*. Other streptococci and lactobacilli commonly employed as starter cultures in dairy products also were included in the study.

Lactic acid bacteria employed as test organisms in various media included: *S. lactis* strains E, 262, and ATCC 7963; *S. cremoris* 12, 101, 127, 144, 169, 208, 275, 300, 306, and ATCC 9596; *S. cremoris* var. *aromaticus* 4, 11, 20, and 22E; *S. thermophilus* S, C-3, and Mc; *S. durans* A; *Lactobacillus acidophilus* Farr; *L. bulgaricus* Gere A; *L. lactis* 39A; *L. casei* ATCC 7469; *Leuconostoc dextranicum* ATCC 8358 and 8086; and *Leuc. citrovorum* 1, 2, and ATCC 8081 and 8082. Four different mixed strain commercial lactic streptococcus starter cultures also were included. Cultures were carried in 10% reconstituted nonfat dry milk solids. Dilutions of culture for plating were made in sterile buffered distilled water to provide plates with colonies in the range of 30 to 300 per plate. Two ml. of dilution were transferred to respective triplicate plates for each organism and test medium.

Various representative commercial dehydrated culture media were studied for basis of comparison or because they had been suggested for certain types of lactic acid bacteria. They included the following Difco products: tryptone glucose beef extract agar (old standard milk plating medium), tryptone glucose yeast extract agar (new standard milk plating medium), tomato juice agar, trypsin digest agar, liver veal agar, micro-assay culture agar, nutritive caseinate agar, whey agar, and stock culture agar. Baltimore Biological Laboratory dehydrated media included: milk protein hydrolyzate medium (new standard milk plating medium), lactalysate agar, eugon agar, trypticase soy agar, trypticase sugar agar, and lactobacillus selective medium. Dehydrated Lstock culture agar of Albimi Laboratories and Sunkist Growers' orange serum agar No. 77 also were used.

The following media reported in the literature were compounded in the laboratory according to the authors' instructions and tested for their ability to support colony development of the lactic acid bacteria: Briggs tomato juice agar, Evans and Niven APT agar, carrot-liver agar, Fabian's V-8 vegetable juice agar, and Hunter's medium.

The reference or test medium with which all others were compared represented a modification of McLaughlin's triple sugar agar (*1*). The modified medium has been employed in this laboratory for both teaching and research purposes and has proven superior to all others tested from the standpoint of ease of preparation, clarity and ease of counting, and colony

development of lactic streptococci and most other lactic acid bacteria.

It has been useful not only for lactic streptococci but also for other lactic acid bacteria found in dairy products, as well as for bacterial counts on silage and other materials supporting lactic fermentations. The new medium has been termed "lactic agar" and has the following composition: tryptone, 2%; yeast extract, 0.5%; gelatin, 0.25%; dextrose, 0.5%; lactose, 0.5%; sucrose, 0.5%; sodium chloride, 0.4%; sodium acetate, 0.15%; ascorbic acid, 0.05%; agar (Noble), 1.5%; and reaction pH 6.8 before sterilization.

Space does not permit enumeration of results with individual media and the numerous variations of the experimental medium from which the final lactic agar was evolved; however, it might be mentioned that most of the media reported in the literature appeared to be designed for specific groups of lactic acid bacteria and few supported growth of all strains of the lactics tested. The lactic streptococci appeared to be particularly fastidious in this respect. Some media recommended for lactobacilli produced no growth or poor colony development of some lactobacillus species. Media that provided excellent to very good results with the organisms tested are listed as follows in descending order of effectiveness: Briggs medium, Fabians' V-8 vegetable juice agar without the brom cresol green indicator, orange juice serum agar with reaction adjusted upward to pH 6.8, trypticase sugar agar, and L-stock culture agar.

Some latitude is possible in composition of the lactic agar. Replacement of 0.5% of the tryptone in this medium with 0.5% sodium caseinate provided excellent growth and aided in detection of acid production and protein hydrolysis by colonies. Tryptone was selected as principal nitrogen source on basis of uniformity and growth-promoting ability. Other protein products that appeared to provide suitable growth included: Bacto-casamino acids, Bacto-peptonized milk, BBL phytone, BBL trypticase and BBL polypeptone. Definite stimulation of growth and increase in colony size of a number of lactics was provided by inclusion of gelatin which is an excellent source of glycine, an amino acid present in low concentration in casein hydrolyzates. Yeast extract also stimulated colony development of some organisms tested.

Various combinations of only two sugars or one sugar alone in some instances showed poorer growth than when all three sugars were included. Some strains of *S. cremoris* var. *aromaticus* showed slightly better growth on media containing glucose alone. Media lacking lactose were poor for some species, as for example *S. thermophilus*. Old milk plating agar was superior to the new in this respect.

Number and size of colonies of lactic streptococci were greater when sodium chloride and sodium acetate were added to the lactic medium. Substitution of potassium for sodium salts did not increase number or size of colonies. Ascorbic acid did not affect lactic streptococci but stimulated growth of some lactobacilli, in particular *L. bulgaricus*.

Omission of agar from the lactic medium provided an excellent broth medium for all types of lactic acid bacteria employed in these studies. Noble's agar was included instead of flake agar primarily because it resulted in greater clarity of medium and thus facilitated colony counting and examination.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

The Control of Rancidity in Milk[1]

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During the past few years the problem of rancidity in milk has been growing in importance, and many people have become interested in learning more about it. Unfortunately, the literature contains much that seems contradictory. Paul Sharp used to complain bitterly that there are two kinds of facts: true facts, and facts that ain't so; and it is very hard to tell which are the "true facts." It might be better to describe the situation somewhat differently. There are two kinds of facts. Some of them are always true but many of them are only true under special circumstances. It is not easy to tell which are which; and it is even more difficult to recognize the special conditions which determine whether a fact is true or not true.

B. L. Herrington

What do we mean when we speak about rancid flavors? The word means different things to different people. Outside of the dairy industry, the term is applied to all forms of fat deterioration. When someone is talking about rancid lard, or cottonseed oil, he usually means the material is oxidized. In the dairy industry, we try to restrict the term to flavor defects resulting from hydrolysis; the reaction of fat with water. The products formed are free fatty acids, di- and monoglycerides, and possibly free glycerine.

The short-chain acids are responsible for the odor of rancid milk. Their odor is much more apparent when the sample is hot. It may escape notice if milk is examined when it is very cold.

The odor of rancid milk is not always the same. Sometimes the odor of butyric acid is easily recognized but sometimes the odor is described as dirty, or as goaty, which suggests that acids of 6, 8, and 10 carbon atoms are more abundant. Many years ago Dorner and Widmer commented that the rancid flavor of homogenized milk was different from the rancid flavor of milk which became rancid spontaneously. If this observation is true, it deserves more investigation for it might provide an important clue to the nature of lipase action. In the meantime, one should not expect that all rancid milk will smell exactly alike; there seems to be some variation in the relative amounts of the acids set free.

In addition to the rancid odor, rancid milk may have a bitter taste. The origin of this is unknown. It may be due to mono- or diglycerides. It may be caused by a rearrangement of fatty acids in the glyceride molecule. It has been reported that glycerides containing butyric acid in the alpha position have a bitter taste. If this is a "true fact," it would indicate that in milk fat the butyric acid is in the beta position. Some people are much more sensitive than others to bitter tastes. Some people miss this bitter flavor entirely.

Rancid milk and cream have another characteristic which may best be described as a "feel." This develops slowly, leaving a puckering sensation in the mouth and an irritation in the throat. This may induce coughing, but not until a short time has elapsed.

This description of rancid flavor has been presented in detail to aid in its identification. It may be described more briefly in the words of a man who complained about the cream on his hot oatmeal: "It stinks."

We believe rancidity in milk is caused by an enzyme because it can be prevented by heating the milk. Pasteurization, as defined by law, is adequate to control lipase action in market milk and cream. It may not be adequate to prevent rancidity in cream that is to be stored for a long time. However, it is customary to use higher temperatures when pasteurizing cream for storage in order to minimize oxidation, and that automatically gives increased protection against lipase.

If you read the literature to learn what time and temperature is actually needed to destroy lipase, you will find many conflicting reports.

[1] This paper was part of a symposium given at the annual meeting, June, 1956.

Part of the discrepancies may be traced to the fact that different tests were used to detect lipase action, and some tests are more sensitive than others. This may not be the entire answer, however. A year ago at the meeting in Michigan, we were told that even a few parts per million of ferric iron protected lipase from destruction during normal pasteurization.

Rancidity in milk is caused by an enzyme. Strangely enough, this enzyme is present in all mixed raw milk. That seems hard to believe because most milk samples do not become rancid during normal storage, but we can prove that lipase is present by homogenizing the milk. So far as is known, all samples will become rancid if treated in this way, though, of course, not all samples have been tested. Pasteurization will prevent this, and that is considered good evidence that an enzyme is responsible for the rancidity induced by homogenization.

All milk seems to contain lipase. We have reason to believe that a considerable quantity is present because if only a small amount of raw milk is mixed with homogenized pasteurized milk, the mixture will usually develop a rancid flavor. Larsen, Gould, and Trout used a sample of raw milk that showed no rancidity after 10 days' storage. However, when this milk was diluted with 4 parts of homogenized pasteurized milk, the mixture became rancid in one day. Failure of the original milk to become rancid in 10 days cannot be explained in terms of lack of enzyme.

Milk lipase is usually considered to be inactive in the udder. Our friends in Ohio have presented indirect evidence that some lipolysis may occur in the udder, but the writer has heard of only one case where milk was actually rancid at the time of milking.

If milk is drawn from the udder and held warm, the lipase remains relatively inactive. When the milk is cooled, lipase action is accelerated. Rarely, the increase in activity is enormous. The milk may become unmarketable in a few hours. Usually, the effect of cooling is much smaller, though some samples may become rancid in 24 hours. In the majority of samples, rancidity cannot be found by taste even after several days. Nevertheless, chemical analysis can show clearly that lipase action is proceeding slowly in the cold milk.

This phenomenon of activating lipolysis by cooling needs more study. It is probably responsible for the fact that lipolysis in milk occurs more rapidly under refrigeration than at room temperature. We sometimes describe this fact by saying that lipase action in normal milk has a negative temperature coefficient. Something has a negative temperature coefficient, but we are not sure what it is.

Incidentally, when lipase acts upon homogenized milk fat, or some other artificial substrate, a positive temperature coefficient is always observed. The negative temperature coefficient is found only when studying fat globules with their natural surface "membrane." This fact suggests that the properties of the globule membrane play an important role in governing lipase activity.

In exceptional cases, milk samples become rancid within a few hours after cooling. No practicable method of preventing this is known except by heat treatment. When spontaneous rancidity occurs in the mixed milk of a herd, it may be possible to identify the cows responsible by taking individual samples from each cow. The samples should be refrigerated for 24 hours and then examined for taste and odor. If the trouble can be traced to only a few cows, their milk should be excluded from the supply. If the milk could be heated quickly enough, it might be saved. For this purpose, pasteurization would not be needed. Merely heating to 145° F. and recooling would probably give sufficient control until the milk could be pasteurized.

Milk that becomes rancid quickly as a result of cooling alone has been called "spontaneously rancid milk." It has been reported that lipase action in such milk can be checked by diluting it with a relatively small volume of normal milk. This is contrary to the writer's experience, but perhaps he has not had the right kind of normal milk for dilution. More evidence is needed concerning this phenomenon.

In most samples of raw milk, lipase action is so slow that it cannot be detected by taste even after many hours of storage. Nevertheless, rancidity may develop if the milk is subjected to certain treatments which are said to "activate the lipase." It might be more correct to say that we activate the fat globules, though recent experiments by W. J. Harper make that less certain. The truth is simple. We don't really know what happens except that certain treatments will cause the milk to become rancid much more quickly than it would otherwise.

Activation by homogenization is the most effective means of activating lipolysis. The violent shaking of warm milk has a similar effect, on a smaller scale. Butter churned from raw sweet cream is likely to become rancid. This does not happen in sour cream because most of the lipase is inactivated by the acid.

If cold raw milk is warmed to 30° C. and recooled, the speed of lipase action is greatly increased. We can only guess at the reason for this. The upper temperature seems critical. Heating to higher or lower temperatures than the critical point has much less effect. It may be significant that this critical temperature is the one at which the fat is almost but not quite melted. It is of interest, also, that the temperature cycle that produces the maximum activation of lipase is the same cycle as that used to increase the body of cream.

Many have asked if temperature activation occurs when warm milk is added to cold milk in a farm tank. We have no evidence that it

does occur where milk is added continuously to the tank during milking. Under these conditions, it seems doubtful that much of the milk is warmed to a high enough temperature to cause trouble. The situation might be different if a large amount of warm milk were added to the tank at one time. It would seem good practice to add the milk to the tank as fast as it is drawn from the cows and to be sure that the refrigeration and agitator are turned on before adding warm milk.

It might be added that homogenized milk does not show temperature activation. This is more evidence that the globule membrane plays an important role in controlling lipase activity in milk.

In recent years a number of pipeline milkers have been installed in New York State. Many of them have vertical sections in the line, which are called risers. The milk is carried up these risers by a large volume of air passing through the line. The first time the writer saw a pipeline milker in operation, he predicted that the churning of milk in these risers would aggravate the rancid milk problem. Experience has confirmed this, not only in New York but in other states as well. Milk handled in a pipeline without risers is more likely to become rancid than milk handled in buckets, and the risk of rancidity is greatly increased if the pipeline contains one or more risers. In studies along these lines, great differences were observed in the sensitivity of milk to such treatment. Some milks seemed very sensitive, but others could tolerate a great deal of abuse. Does this mean that there is a difference in the thickness, the flexibility, or the toughness of the film around the fat globules in different milks? We need to know.

One observation was quite puzzling. The milk from a certain herd was very sensitive to processing in a pipeline that had no risers at all and yet that same milk passed through a centrifugal pump without harm. If activation were due to agitation alone, why didn't a centrifugal pump cause trouble in this milk which was so very sensitive? What happened in the riser that did not happen in the centrifugal pump? Only one difference could be found and that was foam formation.

To test this point, one experiment was set up to give a maximum of agitation but no foam, and another experiment was planned to yield foam with a minimum of agitation. For maximum agitation a Waring Blendor was used. It was completely filled with milk to exclude all air. This was operated by a one-quarter horsepower motor which drove the agitator blades at more than 10,000 r.p.m. To produce foaming with minimum agitation, air was bubbled through a column of milk. The results were clear-cut. Foaming, not violent agitation, was associated with the development of rancidity. This seems to be purely a physical phenomenon. The nature of the gas in the foam is not important; air, nitrogen, or oxygen — it makes no difference.

These experiments have been repeated and confirmed by Tarassuk in California. His study is reported in the *Journal of Dairy Science*, **38**: 438, 1955.

It was suspected that the violent agitation in the risers was partially homogenizing the milk and that this was responsible for the development of rancidity. This explanation seemed less likely when it was discovered that the violent agitation of milk in a filled Waring Blendor did not produce rancidity. To study this problem, a special technique was developed to measure the break-up of fat globules. When the Waring Blendor was completely filled, very few fat globules were broken, but when air was admitted and foaming occurred, the number of very small globules increased many fold. This was surprising, but it offers a clue for the study of the effect of foaming upon milk.

The lesson taught by these experiments is relatively simple even though we do not understand the details. We must examine our milking machines, pipelines, and pumps and redesign them whenever possible to keep foaming at a minimum in order to keep lipase action at a minimum.

Foaming is not serious in cold milk. As milk is cooled below 30° C., there is a rapid loss of sensitivity to foaming. This is probably the reason why we have not been able to trace rancidity to the mechanical agitation in farm tanks. Trouble might develop if the agitators were run without refrigeration.

It seems desirable to say a few words about methods of measuring lipase activity in milk. Some people have tried to record numerical values for flavor and odor but they have not all used the same techniques. Rancidity, which might be missed when examining cold milk, can be found more easily if the milk is heated before examination. That should always be done when you are looking for rancidity. Unfortunately, organoleptic analysis is unable to reveal the changes that take place before flavors appear. Tasting gives no warning of trouble before it arrives. For that reason, a number of laboratory methods have been developed for measurement of the fatty acids set free by lipase action. These methods usually involve two steps: first, the recovery of the fat and a part of the free fatty acids; second, measurement of the free acid in the fat. A number of procedures are used to recover the fat for analysis, but they are not equally efficient. We can prepare fat samples by churning. When we do so, we lose most of the water-soluble acids. We can extract the fat with solvents. These recover part of the water-soluble acids, but not all. Nevertheless, fat prepared in this way shows greater acidity when titrated than fat prepared by churning. Some have used detergents to recover the fat, but the materials added to the milk seem to increase the solubility

of the acids in the water layer. Fat prepared in this way contains less acid than that prepared by churning. Each procedure is useful, but their results are not interchangeable. When you report temperatures, you must indicate whether you used the Centigrade or Fahrenheit scales. When you report acid degree values, you should always tell how the fat was isolated for analysis.

There is much that we do not know about lipase action in milk. Much that has been reported seems contradictory because different workers have used different methods to study different milk supplies. We must remember that what is true under one set of conditions may not be true under other conditions.

There should be closer cooperation among those doing research in this field. It would be easier to compare results if we could reduce the number of variables involved. It is probably impossible for everyone to use the same milk, though that would be most desirable. It might be possible to exchange samples of heated milk to compare analytical methods.

There are many problems that need investigation:

Why is milk lipase inactive in the udder?

Why does cooling milk speed up lipase action, and why is there so much variation in the response of different milks to this treatment?

At one dairy plant, Sharp and DeTomasi found a correlation between the chloride content of milks and their lipolytic activity. Is this generally true?

Why do some samples of milk seem much more sensitive to handling in pipelines than others?

Several people have reported that milk fat containing free fatty acids is more susceptible to oxidation. Is there any relation between lipase activity and the susceptibility of milk to oxidation?

How can we measure damage to the fat globule membrane?

What changes occur in the surface membrane on fat globules as a result of warming, cooling, and agitation?

What is the explanation for temperature activation?

How can we isolate and separate the lipases of milk?

It will not be easy to find answers to these questions. Who wants to try?

General Technology of Cheese Manufacture[1]

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The object of the various cheese manufacturing procedures is to establish conditions suitable for the biochemical and physical changes which are responsible for the development of characteristic body and flavor. With over 400 varieties of cheese known, over 400 methods of manufacture are employed for that purpose. However, the treatments by which all of these varieties are brought into their final state, that is just before being placed in the curing room, have much in common for all varieties.

The basic steps for changing milk into cheese are: (1) addition of starters for acid development, (2) curd formation, (3) cutting, (4) firming the curd, (5) draining and pressing, and (6) salting. By variations in the application of the above steps, finished cheese of widely different physical-chemical properties are obtained.

A general coverage of the significance of each step in the cheese-making procedure will simplify the discussion of the individual cheese varieties.

[1] The purpose of this article is to furnish those unskilled in the science of cheesemaking with a better understanding of the basic steps involved in the manufacture of the various types of cheese.

Starter cultures. Starters of lactic acid organisms are used for the majority of cheese varieties. These bacteria develop lactic acid from lactose, thereby lowering pH of the cheese. The starter bacteria most commonly used are *Streptococcus lactis* or *Streptococcus cremoris, Streptococcus thermophilus, Lactobacillus bulgaricus* and *Lactobacillus lactis.* The former two organisms are usually used for cheese where the cooking temperature does not exceed 102-104° F. and the latter organisms are used for cheese where the curd is treated at temperatures beyond 104° F.

Starter is added to the milk some time prior to addition of the coagulant. The amount used and the length of the period before rennet is added depend on the amount of acid and the rate of acid development desired. The proper acid development, or pH, established in the finished cheese prior to curing varies from 4.7 to 5.5, depending upon variety. However, the pH of a given variety generally has to be within $\pm$ 0.1 pH unit of the desired level or inferior cheese will result from off-fermentations.

The majority of the foreign varieties of cheese requires a rather slow acid development compared to Cheddar cheese. For this reason, a smaller amount of starter is used. This

Courtesy of Kraft Food Company

FIG. 1. Hooping the curd for soft types of cheese. The whey helps retain moisture in the cheese and results in a relatively closed texture cheese.

ranges from 0.1 to 1% with an average of about 0.5%. In some cases the cheesemaker depends on naturally present lactic acid organisms to produce the desired amount of acid. This is particularly true where cheese is produced on the farm from which the milk is obtained and where manufacturing methods have not been standardized.

Coagulation. The property of the protein, casein, which permits its coagulation by acid or rennet is a key essential to cheesemaking. Coagulation of the milk or curd formation is usually accomplished by addition of the enzyme, rennin, to the milk. Rennin may be added in the form of an extract, powder, or paste prepared from the stomachs of young mammals, usually calves. The coagulation of casein by rennin is now generally accepted as being a two-step process. The first step involves the action of rennin in causing a proteolytic cleavage in the protein. The second step, which results in coagulation, involves calcium ions, which may act to form a high molecular weight polymer that forms the coagulum.

The amount of rennin, setting temperature, and setting time vary with the type of cheese and are related to the desired curd firmness. The amount used ranges from 1½-4 oz. rennet extract per 1,000 lb. of milk, the temperature from 84° to 92° F., and the time from 20 to 90 minutes. The more rennet, the higher the temperature, or the longer the setting time, the firmer is the resulting coagulum. Comparatively little rennet and short setting time are used for Swiss cheese, for which the curd is cut fine and high cooking temperatures are depended on to firm the curd. On the other hand, more rennet and longer setting time are used for Blue cheese, for which the curd is cut in larger cubes and heat is not used to firm the curd.

Although rennin has been found the most suitable coagulant for the majority of cheese varieties, certain types of cheese, such as Sapsago and Gammelost, use acid as a coagulant. The acid is usually developed by lactic acid bacteria. Curd formed in this manner is distinctly different from rennet-coagulated curd and results in distinct types of cheese.

Cutting. After the coagulum has reached the desired firmness it is cut. The object of cutting is to divide the large mass of curd into smaller, uniform particles in order that moisture may be more easily expelled. For most varieties the common American cheese knives are used whereby the curd is cut into cubes of specific uniform size ranging from 3/16 to 3/4 in. cubes. Special instruments, like the harp and the sabre, may be used for types like Swiss and Camembert, respectively. The modern mechanical vats are equipped with cutting devices, which may use wires strung vertically, diagonally, or in a grid form in a metal frame. These knives are applied mechanically to the curd until desired particle size has been obtained.

The cutting of the curd is the initial step in controlling the moisture content of the finished cheese. The retention of moisture in the curd is directly related to the size of the cut. Examples of different sized cuts are Camembert, which may not be cut at all or only

Courtesy of Kraft Food Company

FIG. 2. Blue cheese curd is drained of free whey before hooping. This results in an open texture essential for this type of cheese.

Courtesy of Olson Publishing Company

FIG. 3. When eye formation is desired, the curd is allowed to mat under whey to form a close texture free from mechanical openings. After matting, the curd is cut in blocks of desired size before hooping.

in large, irregular chunks; Blue cheese and Tilsit represent types with a medium cut of about 3/8-1/2 in. cubes; and Swiss and Parmesan curd is usually worked until the individual particles are the size of wheat grains.

Firming the curd. Firming of the curd in general can be divided into three phases — preworking, heating, and cooking. The moisture content of the finished cheese is controlled by utilizing various combinations of these three factors.

During preworking the curd is agitated gently just enough to keep it from matting. Excessive agitation will break the curd particles and result in lower yields and excessive fat losses. Cheese in which a high moisture content is desired may be firmed entirely by preworking, and in this case no heating is used. The curd may be allowed to rest undisturbed from 0 to 30 minutes before hooping, or the curd may be agitated intermittently for 1 to 2 hours and then hooped.

When heat is used, the curd is preworked 10-20 minutes, then agitation is increased and heat applied slowly. The desired temperature should be reached in 20-50 minutes depending on the type of cheese being made. Too rapid application of heat may result in lumping and formation of a film on the surface of the curd particles, preventing proper expulsion of whey. After heating, the curd is cooked, usually under constant agitation, until desired firmness has been reached.

Gouda, a firm-bodied cheese, is heated to 100-104° F., and the entire treatment may take 1-1½ hours. Parmesan, a very firm-bodied cheese, may be heated to as high as 125-135° F., and the firming process lasts about 2 hours.

During the firming process, the curd particles contract and whey is expelled from the curd. The contraction is brought about by both acid formation and heat. The types of cheese that are not heated beyond the setting temperature rely mostly on acid development for whey expulsion. With cheese heated to 95-104° F., the firming of the curd is the combined effect of acid development and heat. Cheese heated to 115-130° F. is almost entirely firmed by heat, since very little acid is developed prior to pressing.

Heat can be applied either by indirect steam or by withdrawing part of the whey and replacing it with warm or hot water. The method employed depends on the body and flavor characteristics desired. Heating by addition of warm water results in cheese with a softer and more elastic body and the cheese has a tendency to be more susceptible to off flavors than that of comparable cheese heated by indirect steam. The reason for this is that the water washes out part of the lactose, resulting in less total lactic acid being formed.

Whey removal. When the curd has reached the desired firmness, the whey is removed. The curd is then hooped and pressed. High moisture cheese, such as Port Salut, usually is dipped out of the vat into the hoop along with some whey. The harder varieties are usually dipped with as little whey as possible. For stirred curd varieties, such as Gouda, where mechanical openings are a characteristic of the cheese, the whey is removed completely before the curd is hooped as individual particles. For varieties where eye formation is desired, like Samsoe, the curd is allowed to mat and is assembled under whey and removed in one solid mass, resulting in a tightly knitted cheese.

After the curd is hooped, pressure may be applied. Some soft cheeses like Camembert or cheeses with an open texture like Blue and Tilsit are not pressed. The amount of pressure applied on pressed cheese varies from 1 to 3 lb. on Limburger to 2,000-4,000 lb. on Swiss.

The object of pressing is to aid in removal of whey from the curd and to give the finished cheese a closed surface. A bandage or cloth is usually placed around the cheese.

Salting. Salt is applied to most varieties of cheese to control undesirable fermentations. In some varieties part or all of the salt may be added at some time before hooping. In general, the penetration of the salt into the curd is inversely proportional to the time between dipping and salting.

When salting is applied during the making of the cheese, the purpose is to firm the curd and to slow down or, in extreme cases, even prevent further activity of the starter organ-

isms. However, most varieties are salted after pressing. The prime purposes of salting at this stage are to firm the cheese so it will retain its shape and to control fermentations during curing. For example, proteolysis is favored by low salt content. Cheeses like Bel Paese and Port Salut, where the flavor is due primarily to protein breakdown, are low in salt. Cheeses like Swiss and Romano, where less extensive proteolysis is desired, are high in salt.

Brine salting and dry salting or a combination of the two are the methods used for salting the finished cheese. Of these, brine salting is the more rapid method and is usually applied to cheese where some rind formation is desired and is always used for larger cheese because of the ease of handling and the extra amount of time that would be involved in applying dry salting. The cheese is placed in a nearly saturated brine solution at approximately 50° F. The length of time of submersion depends on the desired salt concentration in the finished cheese, the size of the cheese, and the concentration and temperature of the brine.

Dry salting is accomplished by distributing salt over the surface of the finished cheese, repeating at 24-hour intervals as many times as needed, depending on the desired salt content of the cheese. Dry salting is a relatively slow and mild treatment and is usually applied to cheese where little or no rind is wanted.

Gestation Period for Holstein-Friesian Cows

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Holstein-Friesian Association of America, Brattleboro, Vt.

Over the years many studies have been made of the period of gestation in cattle and the literature contains many references to these investigations, several dating back more than a century. Some of these reports deal primarily with the average length of the period the cow carries her calf, and others have considered the effect of breed, sex, age of cow, season of year in which birth occurred, singles as compared to twins, etc. A gestation table is very convenient for the cattle breeder in determining as closely as possible the expected date of parturition in order that the cow may be properly prepared. Many firms dealing with livstock breeders supply their customers with gestation tables, and many textbooks devoted to livestock breeding include gestation tables for different species of farm animals.

Feeds and Feeding, by Morrison, 20th ed., 1936, says, "The average gestation period of cows is placed by most writers at 280 to 285 days; however, in studies of the gestation periods for cows of the leading dairy breeds, the average period has been only 278 to 280 days."

Dairy Cattle and Milk Production, by Eckles, 1939, gives a gestation table for cattle based on 282 days.

Dairy Science, by Petersen, 1939, gives 281 days as the average gestation period for cows with about equal chances for calving on any day from the 275th to the 287th day after service.

Breeding and Improvement of Farm Animals, by Rice, 1942, gives a gestation table for cattle based on 281 days.

Dairy Cattle Breeding, by Gilmore, 1950, gives a gestation table for dairy cattle in which breed differences are recognized: Holsteins, Ayrshires and Jerseys are based on 278 days; Guernseys, 283; and Brown Swiss, 289 days.

Detailed reports of the different studies that have been published would require considerable space, and this discussion is confined to summaries of the findings with Holstein-Friesians and brief reports of some of the conclusions.

One of the most extensive studies was made by J. C. Knott, State College of Washington, *Journal of Dairy Science,* **15**: 87. 1932. The data used were obtained from Carnation Farms, Western State Hospital, and Washington State College Holstein herds. All gestation periods terminated by the birth of living calves were included, except those where the breeding records were marked to show an abnormal calving. The average length of 2,824 gestation periods that terminated with single births was 279.9 days. In these studies both terminal dates, that is, the date of service and the date of birth, were included. These 2,824 cases ranged from 262 days for the shortest to 296 for the longest with 1,273 or 45.1% from 270 to 279 days, inclusive, and 1,427 or 50.5% from 280 to 289 days, inclusive.

The distribution chart showed fewer calves born the 280th day (the mean) than on the 279th, 281st, or 282nd day. The average gestation period for 1,441 male calves in this study was 280.4 days as compared with an average of 279.4 days for 1,383 female calves. These studies revealed 86 sets of twins not included with the figures above, for which the average gestation period was 275.5 days, or 4.4 days less than for the single births. The 86 sets of twins included 24 male and male, 42 male and female, and 20 female and female.

A study of "Factors associated with the duration of gestation in dairy cattle," by Brakel,

Rife and Salisbury of Ohio State University was published in the *Journal of Dairy Science*, **35**: 179. 1952. The data used in these studies were compiled from the records of the Ohio State University purebred dairy herd from 1922 to 1949. These records included 354 Holstein calves born singly, and the average length of gestation was 278.57 days, not counting the date of service, with a range from 260 days for the shortest to 299 days for the longest period. There were 149 or 42.1% of the total carried from 275 to 279 days and 94 or 26.5% of the total carried from 280 to 284 days. Male calves were carried 0.77 day longer than females and twins were carried 3.4 days less than calves born singly.

Other data, some of which have not been published, include a report from Dr. Rice, University of Massachusetts, showing an average of 278.2 days for 50 cows in the University Holstein herd. Heizer gives the following data from the University of Wisconsin Holstein herd:

185 heifer calves carried 275.6 days
170 bull calves carried 277.9 days
355 calves, both sexes, carried 276.7 days

Yapp and Alexander report for the University of Illinois Holstein herd:

337 female calves carried 279.9 days
329 male calves carried 281.2 days
666 calves, singles of both sexes carried 280.4 days

Other interesting observations are reported in some of these studies. Knott found that the gestation period increased with the age of the dam up to 6 years and then tended to decrease slightly. He found that the length of gestation was approximately 1½ days longer for 6-year-old cows than for 2-year-olds. This was corroborated by Brakel at Ohio. Both Knott and Brakel found a relationship between the time the cow herself was carred in dam and the length of gestation of her calves. Brakel reports that spring-calving cows carried their calves 2 days longer on the average than cows calving in the fall.

For many years the Holstein-Friesian Association of America published and used a gestation table based on 283 days. This was changed in 1951 to 279 days and since then we have had some inquiries and objections from breeders who felt that the longer period was more nearly correct for the breed. The change was made on the basis of the many studies which we have reported in this article, but it appeared desirable to make some studies of our own records for comparison with the published data.

We decided to use the applications for registry which had been filed by the owners of ten well known and widely scattered herds, each using a prefix name which enabled us to locate them readily from the alphabetical index in the herdbook. The original registry applications are held for approximately 5 years before destroying after they have been photographed on recordak film. Since for our special purpose it was much easier to work with the applications, the studies were made from applications filed during the past 5 years. In these selections only applications which showed both terminal dates were used; applications for calves resulting from pasture service and applications for re-registration of calves recorded in the Canadian Herdbook were rejected because the service date was not available. Service and birth dates on the application were compared with our regular gestation table of 279 days, which includes both service and birth dates, and the gestation period for each calf was determined. Applications were kept separate according to sex and owner, and twin births were considered separately.

Applications examined from these ten herds included:

1,193 females, born singly and carried 333,244 days averaging 279.4
760 males, born singly and carried 213,383 days averaging 280.8
1,953 calves of both sexes carried 546,627 days averaging 279.9

Gestation periods of these 1,953 calves varied from 260 to 305 days and 73% were born within the 11-day period, 275-285 days after date of service. Births of 33 twins recorded showed an average gestation period of 275.1 days, or 4.8 days less than the 279.9 day average for the 1,953 single calves.

To supplement these figures we decided to take a random sample of approximately 1,000 male calves, born singly, and an equal number of females. For this group we selected every 10th male recorded, starting with No. 1,085,500 and every 25th female starting with No. 3,417,-500. The random sample applications included 1,106 single born females, 959 single born males, and 29 twin calves (11 females and 18 males).

1,106 females, born singly and carried 310,236 days averaging 280.4
959 males, born singly and carried 270,092 days averaging 281.6
2,065 calves, male and female, carried 580,328 days averaging 281.0

The 29 twin calves in the random sample group averaged 275.6 days, or 5.4 days less than the 281 day average for the 2,065 single born calves. Gestation periods of the 2,065 single born calves, males and females, ranged from 260 to 300 days, and 77.6% were born within the 11-day period, 275-285 days after date of service.

Combining the two groups, those selected from the ten herds and those selected at random, we have the following figures for 4,018 calves, including 2,299 females and 1,719 males, all single born calves:

2,299 females were carried 643,480 days, an average of 279.9 days.

	No. of gestation periods	*Average No. days*
Wing (Cornell) 1899	97	280 (?)
McCandlish (Iowa) 1922	111	278 (?)
Fitch (Kansas) 1924	220	281 (2)
Knott (Washington) 1932	2824	279.9 (2)
Kucop (Ohio) 1934	432	278.8 (1)
Henderson (Cornell) 1938	437	279.8 (?)
Herman (Missouri) 1947	962	278.1 (1)
Alexander (Illinois) 1950	666	280.4 (?)
Heizer (Wisconsin) 1951	355	276.7 (?)
Brakel (Ohio) 1952	354	278.6 (1)
H.-F. Ass'n. of America (1956)	4018	280.5 (2)

(?) Count of terminal dates not reported

(1) Birth date only counted

(2) Both terminal dates included in count

1,719 males were carried 483,475 days, an average of 281.2 days.

4,018 males and females were carried 1,126,955 days, an average of 280.5 days.

For convenient reference we present the following table which includes most of the studies reported for Holsteins and indicates whether one or both terminal dates were included in the count, if the report included this information.

Summary figures for several studies of length of gestation period for Holsteins are given in the opposite column.

These summary figures from 11 separate studies show as close agreement as might be expected. In three studies where both terminal dates were included the averages were 279.9, 280.5 and 281 days. The three studies which included only the date of birth averaged 278.1, 278.6, and 278.9 days, and the other five where the method of counting was not reported ranged from 276.7 to 280 days. In the studies which included the largest numbers, Knott found an average of 279.9 days for 2,824 calves, and figures for 4,018 calves taken from the registry applications in the office of the Holstein-Friesian Association of America showed an average of 280.5 days.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

643. Currents in Biochemical Research 1956. Edited by DAVID E. GREEN. Interscience Publishers, Inc. 697 pp. $10.00 (1956).

Currents in Biochemical Research is composed of 27 essays on biochemical subjects. The authors are recognized specialists writing about problems with which they have made significant research contributions.

In the manner of Volume I the contributors to the 1956 edition attempted to make a survey (for the most part a decennial survey) of "progress in most, although by no means all important fields of biochemical research." "The objectives of these essays have been to communicate to nonspecialists an over-all impression of the present status of the significant problems in each field, to point up the broad strategy of current research, and finally, to speculate on the likely paths of future research."

As one might expect, some topics are treated in a highly specialized manner while others are readily appreciated by the "nonspecialist." It is noteworthy that the most significant contributions of the volume appeal not to the "nonspecialist" but to the active experimentalist. This is not surprising as the editor requested specialists to elaborate on their own problems. In spite of the hazards involved in singling out specific contributions from such a distinguished array of authors one might ask if such chapters as Enzyme-Substrate Compounds and Electron Transfer, and Enzyme Kinetics, both of which are masterfully done, are written for the nonspecialist. Space does not permit a discussion of the 27 individual chapters. As the annual reviews often tend to become a collection of references, this more philosophical appraisal of the problems of biochemistry has a distinctive function.

Many readers will recognize important areas not covered by the volume. Expansion of the scope to encompass other areas of research should be encouraged. The chapters and authors are listed below:

Chemistry and Viral Growth by A. D. Hershey; Photosynthesis by J. A. Bassham and M. Calvin; Bacterial Fermentations by H. A. Barker; Some Aspects of Vitamin and Growth Factor Research by Esmond E. Snell; The Significance of Induced Enzyme Formation by S. Spiegelman and A. M. Campbell; Certain Problems in the Biochemical Study of Disease by DeWitt Stetten, Jr.; The Hormones, Their Present Significance, Their Future by Gregory Pincus; Problems of Cellular Biochemistry by Carl F. Cori; Enzymes as Reagents by Efraim Racker; Attempts at the Formulation of some Basic Biochemical Questions by Fritz Lipmann; Enzyme Complexes and Complex Enzymes by Henry R. Mahler; Relations between Prosthetic Groups, Coenzymes and Enzymes by Hugo Theorell; Enzyme-Substrate Compounds and Electron Transfer by Britton Chance; On the Nature of Hemoprotein Reactions by Philip George; Aspects of Protein Structure by Barbara W. Low and John T. Edsall; The Structure of Insulin by F. Sanger; A New Concept of Ribonucleic Acids by Waldo E. Cohn; Chemical Structure as a Guide to the Study of Biochemical Syntheses by Konrad Bloch; The Role of Nucleotides and Coenzymes in Enzymatic Processes by Frank M. Huennekens; The Biosynthesis of Porphyrins; The Succinate-Glycine Cycle by David Shemin; Problems in the Study of Multiple Enzyme Systems by G. Robert Greenberg; Enzyme Kinetics by Robert A. Alberty; The Interconversion of Sugars in Nature by Luis F. Leloir; A Theory of the Primary Event in Muscle Action by Manuel F. Morales and Jean Botts; Trends in Biochemistry of Nerve Activity by David Nachmansohn and Irwin B. Wilson; Blood: Some Functional Considerations by Douglas M. Surgenor; An Integrated Concept of Carcinogenesis by Harold P. Rusch. R. G. Hansen

644. Process Cheese Abstracts. D. M. IRVINE and W. V. PRICE, Dept. of Dairy and Food Ind., Univ. of Wisconsin., Madison. 1955.

The literature pertaining to process cheese, process cheese food, and process cheese spread, has been reviewed, abstracted and published in a mimeographed booklet of 71 pages.

The references have been classified under two general headings with several subdivisions as follows:

I. Processed Cheese and Related Products.
- A. General Information Dealing with Processed Products
- B. Analysis and Chemical Composition
- C. Body and Texture
- D. Cheese Types Used in Processing
- E. Defects—Physical and Chemical
- F. Emulsifiers and Stabilizers
- G. Equipment
- H. Ingredients—Non-cheese
- I. Methods of Processing and Manufacturing
- J. Packaging
- K. Preservatives
- L. Spreads
- M. Spoilage and Bacteriology

II. Patents of Processed Cheese and Related Products.
- A. Cheese Food Products
- B. Emulsifiers
- C. Process Equipment
- D. Process, Packaging
- E. Preservatives

The references are arranged alphabetically by authors under each sub-division.

The publication is an excellent reference for those who are working with process cheese.

S. L. Tuckey

CHEESE

645. Cheese making apparatus and method. C. Cohen and M. L. Cohen (assignors to Chain-O-Lakes, Inc., Waupaca, Wis.). U. S. Patent 2,761,376. 7 claims. Sept. 4, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 1: 65-66. 1956.

A cylindrical shaped telescoping type of cheese hoop is described. R. Whitaker

646. Cheese packing. C. Pauly. U. S. Patent 2,760,430. 1 claim. Aug. 28, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 4: 818. 1956.

Details are given regarding the construction and assembly of a box for holding cylindrical shaped cheese during storage and shipping. R. Whitaker

647. Cheese cutting machine. B. T. Hensgen and W. H. Beland (assignors to Swift and Company, Chicago, Ill.). U. S. Patent 2,760,248. 15 claims. Aug. 28, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 4: 772. 1956.

A device is described for cutting large blocks of cheese into retail size portions. R. Whitaker

648. Apparatus for producing individually wrapped cheese slabs. A. N. Nawrocki (assignor to Clearfield Cheese Company, Curwensville, Pa.). U. S. Patent 2,759,308. 2 claims. Aug. 21, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 3: 535. 1956.

A thin sheet of process cheese is extruded between two sheets of flexible wrapping material which are continuous heat sealed along the edges. The flow is uniformly periodically interrupted to permit heat sealing across the strip to complete the rectangular shaped package. R. Whitaker

649. Processing of milk products. J. P. Jensen. U. S. Patent 2,758,925. 17 claims. Aug. 14, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 2: 420. 1956.

Dairy products such as cheese, butter, casein, dried milk, condensed milk, etc. are made with a small amount of glycerol and either an acid or a base. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

650. Whipping products and process of manufacture. N. E. Rodgers, R. G. Henika, and R. E. Miersch (assignors to Western Condensing Company) U. S. Patent 2,765,232. 9 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 123. 1956.

Whey is deproteinated with a nontoxic soluble iron salt at a concentration of 0.0015 to .01 molar, within a pH range of 3.0 to 4.7 and at a temperature ranging from 65 to 95° C. The remaining serum has desirable whipping and foam stabilizing properties. R. Whitaker

651. Dry soluble casein mix and method of preparing same. H. S. Elden (assignor to National Gypsum Company, Buffalo, N. Y.). U. S. Patent 2,758,034. 10 claims. Aug. 7, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 1: 176. 1956.

A dry paint or cement mix, consisting of casein and an ammonium salt, which when mixed with water, has a pH of 6.0 to 9.0. R. Whitaker

DAIRY BACTERIOLOGY

652. Thermal resistance of food poisoning organisms in poultry stuffing. R. C. Webster and W. B. Esselen, Dept. of Technol., Univ. of Mass., Amherst. J. Milk and Food Technol., **19**, 209. 1956.

The thermal death time of *Salmonella enteriditis*, *Micrococcus pyogenes* var. *aureus* and *Streptococcus faecalis* was studied in poultry stuffing. A temperature of 165° F. reached in the center of the stuffing during the roasting destroyed the test organisms. *S. faecalis* exhibited a much higher degree of heat resistance than *Micrococcus* and *Salmonella*. It was concluded that 165° F. was satisfactory to destroy food poisoning organisms. H. H. Weiser

DAIRY CHEMISTRY

653. The hydrogenation of dietary unsaturated fatty acids by the ruminant. R. REISER and H. G. R. REDDY, Dept. of Biochem. and Nutrition, Texas Agr. Expt. Sta., College Station. J. Am. Oil Chem. Soc., **33**, 155. 1956.

Goats were fed alfalfa meal containing 10% cottonseed or linseed oil. After 11 weeks the fatty acids of rumen, stomach, and caecum contents were compared to those of the feed. The high levels of linoleic and linolenic acids of the feed were reduced to very low levels in the rumen, with comparable increases in the saturated acids. Monoethenoid acids were increased after linseed oil ingestion and in one animal after cottonseed oil ingestion.

F. A. Kummerow

654. The chemistry of polymerized oils. V. The autoxidation of methyl linoleate. H. H. SEPHTON and D. A. SUTTON, Natl. Chem. Research Lab., Pretoria, South Africa. J. Am. Oil Chem. Soc., **33**, 263. 1956.

The geometrical forms obtained during autoxidation of methyl linoleate at ordinary temperatures are largely conjugated *cis-trans* and *trans-trans* as shown by previous workers. There is a possibility that conjugated *cis-cis* forms also are produced. The *trans-trans* molecules arise partly, at least, by thermal rearrangement of already formed *cis-trans* peroxide. Some proportion, at least, of the *cis-trans* molecules have their *trans* double bonds nearest to the hydroperoxide group. F. A. Kummerow

655. Adapting some official methods to the semi-micro range. R. P. A. SIMS and B. STONE, Can. Dept. of Agr., Ottawa, Ontario. J. Am. Oil Chem. Soc., **33**, 287. 1956.

Occasionally only a limited amount of material is available for analysis and although chromatography could be used to determine composition, conventional indices are required. This situation can be met by extending the range of established micro-techniques or by adapting official methods to smaller sample size. The latter alternative was taken to determine oil content, iodine value, saponification and neutralization equivalents, polyunsaturated fatty acids, and solid fatty acids.

F. A. Kummerow

656. A paper chromatographic procedure for separating 1-mono-, 1,3-di and triglycerides, cholesterol, and cholesteryl esters. J. W. DIECKERT and R. REISER, Dept. of Biochem. and Nutrition, Texas A. and M. Coll., College Station. J. Am. Oil Chem. Soc., **33**, 123. 1956.

A reproducible paper chromatographic procedure has been developed for resolving a mixture consisting of 1-mono, 1-3-di-, and triglycerides, cholesterol, and cholesteryl esters. The separation is accomplished using silicic acid impregnated glass fiber filter paper as the chromatographic medium and various ethyl ether-isooctane mixtures as the solvent systems. A reagent consisting of 1:1 concentrated H_2SO_4:H_2O has been introduced for visualizing the lipides on the chromatogram and for distinguishing between glycerides and cholesterol-containing lipids. F. A. Kummerow

657. The antioxidant properties of some 6-hydroxychromans and 5-hydroxycoumarans. W. K. T. GLEIM and J. A. CHENICEK, Universal Oil Products Co., Des Plaines, Ill. J. Am. Oil Chem. Soc., **33**, 322. 1956.

The inhibitor activity of some 6-hydroxychromans and 5-hydroxycoumarans in gasoline and lard was investigated. The coumarans are the more effective in both substrates. Introduction of a tertiary butyl group ortho to the hydroxyl group increases the activity of 2,2-dimethyl-5-hydroxycoumaran in both substrates, but the activity of the 2,2-dimethyl-6-hydroxychromans is increased only in gasoline.

F. A. Kummerow

658. The trans fatty acids of margarines and shortenings. A. F. MABROUK and J. B. BROWN, Dept. of Agr. Biochem., and Physiol. Chem., Ohio State Univ., Columbus. J. Am. Oil Chem. Soc., **33**, 98. 1956.

Six specimens of margarines and five of shortenings were examined for *trans* fatty acids by infrared absorption at 10.36 μ. With one exception *trans* components calculated as trieladin ranged from 22.7-41.7%; the single example is a specimen of shortening prepared by blending vegetable and animal fats and subjected to minor hydrogenation. The iodine values of these materials were close to the range found for olive oil. The high melting point of the product is due, principally, to the presence of large amounts of *trans* acids.

F. A. Kummerow

659. Periodate-permanganate oxidations. IV. Determination of the position of double bonds in unsaturated fatty acids and esters. E. VON RUDLOFF, Prairie Reg. Lab., Natl. Research Council of Canada, Saskatoon, Saskatchewan. J. Am. Oil Chem. Soc., **33**, 126. 1956.

The reaction of the periodate-permanganate reagent with unsaturated fatty acids, including oleic, elaidic, eicosenoic, 10-undecenoic and linoleic acids, gave the expected end-products in quantitative yields. The latter were isolated and determined by partition chromatography on silicic acid columns in 10 to 100 mgm. quantities. Some compounds, which were unreactive because of insolubility, gave satisfactory results when the medium contained 5 to 10% pyridine. This was true also for methyl linoleate even though solution was incomplete. However, methyl oleate and triolein did not give

quantitative results even in a medium containing 20% or more pyridine in which the esters were completely dissolved. F. A. Kummerow

660. A modified dilatometer for fast evaluation of solids content in fats. B. F. Teasdale and R. Svardal, Canada Packers Ltd., Toronto. J. Am. Oil Chem. Soc., **33**, 78. 1956.

A change was made in the dilatometer design, allowing a substantial reduction in the time required to complete a solids content determination in edible fats. F. A. Kummerow

661. Influence of high energy radiation on oxidation of oleic acid and methyl oleate. L. R. Dugan, Jr. and P. W. Landis, Am. Meat Inst. Foundation, Univ. of Chicago, Illinois. J. Am. Oil Chem. Soc., **33**, 152. 1956.

Gamma radiation from Cobalt 60 influences the oxidation of oleic acid and methyl oleate even at low temperatures. Determination of peroxide values, carbonyl values, and the $E_1^{1\%}$cm values at 224 mμ revealed that high peroxide values could be obtained but that secondary products are formed in appreciable quantities. The products causing absorption at 224 mμ may be α, β-unsaturated ketones. The level of these substances can be increased by irradiation-oxidation in the presence of metal soaps such as cobalt stearate. Irradiation-oxidation of methyl oleate through a series of temperature ranges from 7.5 to 55° C. revealed a marked thermal activation effect. Peroxide values of 2000 me/kg or greater were obtained in 100 hr. of reaction time. F. A. Kummerow

662. Some effects of ionizing radiations on lipids. I. Monocarbonyl production in vegetable oils. D. A. Lang and B. E. Proctor, Dept. of Food Technol., Mass. Inst. of Technol., Cambridge, Mass. J. Am. Oil Chem. Soc., **33**, 237. 1956.

Irradiation of refined vegetable oils by high-energy cathode rays resulted in the production of monocarbonyl compounds. The yield was about 0.2 μM per gram per 10^6 rep. for doses up to about 10^7 rep. Some of the products were themselves sensitive to the radiations, and the net yield was dependent upon a function of the concentrations and reactivities of the precursors and of the end-products.
F. A. Kummerow

663. Separation of monoglycerides, diglycerides, and triglycerides by liquid-liquid extraction. R. E. Treybal and J. A. Monick, Colgate-Palmolive Co., Jersey City, N. J. J. Am. Oil Chem. Soc., **33**, 193. 1956.

Pure mono-, di-, and trilaurin mixtures, and commercial monoglycerides were studied as quaternary systems for monoglyceride distribution with 50 wt.-% aqueous ethanol. Pure and commercial systems showed similar distribution tendencies even with differences in molecular weight and degree of unsaturation. Aqueous ethanol will separate mixtures of mono-, di-, and triglycerides by selective liquid-liquid extraction, however, the literature favors more complex systems based on two immiscible solvents. F. A. Kummerow

664. Concurrent oxidation of accumulated hydroperoxides in the autoxidation of methyl linoleate. O. S. Privett and C. Nickell, The Hormell Inst., Univ. of Minn., Austin. J. Am. Oil Chem. Soc., **33**, 156. 1956.

Kinetic studies showed that concurrent oxidation of preformed hydroperoxides may be expected to take place at all stages of the autoxidation of methyl linoleate. The rate of oxidation relative to the rate of autoxidation of unoxidized ester is determined chiefly by the extent of the accumulation of hydroperoxides. Infrared spectral analysis of hydroperoxides oxidized to various degrees indicated that *trans, trans* diene conjugation and isolated *trans* double bonds produced in the autoxidation of methyl linoleate are related to the concurrent oxidation of the accumulated hydroperoxides.
F. A. Kummerow

665. A rapid method for estimating total protein in milk. D. C. Udy. Nature, **178**, 4528: 314. 1956.

This test is based on the acid-binding capacity of several proteins as measured by the complex which forms when the protein reacts with an anionic dye such as orange G. The proteins of milk have a common binding capacity. The test is conducted as follows: mix 1.5 ml. of milk with 25.0 ml. buffered orange G dye, containing 1/gm/1. Shake 5 min. and centrifuge. The concentration of unbound dye is then measured in a special absorption cell of short light-path using a 470 filter in an Evelyn photoelectric colorimeter and reading to the nearest 0.1% transmission. R. Whitaker

666. Production of lactase. R. P. Myers and E. G. Stimpson (assignors to National Dairy Research Laboratories, Inc., Oakdale, N. Y.). U. S. Patent 2,762,749. 14 claims. Sept. 11, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 2: 426. 1956.

A lactase rich enzyme product is produced by propagating a lactase producing strain of yeast in deproteinated whey in a pH range of 3.5 to 7.5 at 80° and 100° F. and drying the collected yeast. R. Whitaker

667. Drying of yeast to inactivate zymase and preserve lactase. R. P. Myers (assignor to National Dairy Research Laboratories, Inc., Oakdale, N. Y.). U. S. Patent 2,762,748. 3 claims. Sept. 11, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 2: 426. 1956.

To increase the lactase and decrease the zymase content of a lactase yeast preparation it is dried in the frozen state at below 30° F. and under high vacuum. R. Whitaker

668. Process of preparing lactated sugars. H. E. Bode. U. S. Patent 2,762,710. 3 claims. Sept. 11, 1956. Offic. Gaz. U. S. Pat Office, **710**, 2: 419. 1956.

A mixture of about 80% sugar and 20% lactic acid solids and containing less than 20% moisture. R. Whitaker

DAIRY ENGINEERING

669. Some observations on the effect of several organic and inorganic acids in inhibiting the heat precipitation of calcium hardness salts from water. D. A. Evans and G. H. Watrous, Dept. of Dairy Sci., Penn. State Univ., University Park. J. Milk and Food Technol., **19**, 213. 1956.

The precipitation of calcium salts was prevented when water not exceeding 20 grains per gal. of calcium hardness was acidified with gluconic or levulinic acid to pH 5. The acids studied had very little corrosive effect on stainless steel. However, the corrosion effect on other less resistant metals or alloys was not studied.

This procedure involves a saving of time and money in controlling water stone deposits and the accumulation of thermoduric organisms which thrive in alkaline mixture deposited on dairy equipment. H. H. Weiser

670. Apparatus for aerating and agitating milk. W. R. Roop. U. S. Patent 2,760,765. 8 claims. Aug. 28, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 4: 906. 1956.

Milk held for storage in small lots is agitated by means of bubbles released from a tube submerged in the tank. The tank of milk is maintained under sufficient sub-normal pressure to cause the bubbles to rise and provide thorough mixing. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

671. A formula basis of pricing fluid milk in Georgia. J. C. Purcell, J. C. Elrod, and N. M. Penny. Georgia Agr. Expt. Sta. Bull. N.S. 21. 1956.

An explanation is given of the indices recommended in establishing fluid milk prices. Methods of computation also are presented as are the obvious advantages and disadvantages of formula pricing at both retail and wholesale levels. A. W. Rudnick, Jr.

672. Quantity discount pricing of fluid milks. J. D. Helmberger and E. F. Koller. Minn. Agr. Expt. Sta. Bull. 433. 1956.

A survey of Twin City milk consumers and milk distributors, showed that a 10¢ quantity discount on home delivery resulted in a 15% volume increase. These data were confirmed in a southern Minnesota town where a 16% increase in retail sales resulted from an 8¢ quantity discount, and in Des Moines, Iowa, where a 10¢ quantity discount resulted in a 20% increase. It is pointed out that while even 10¢ is not sufficient to pay the actual cost of each home delivery, it is sufficient to cause small consumers to more equitably bear their share of delivery service. It will also cause such consumers to change their buying habits to take advantage of the discount. A. W. Rudnick, Jr.

673. Milk pricing under public regulation in the United States. A selected bibliography. O. Cummings; Library, Div. of Agr. Sci., Berkeley, Calif. 1954.

An extensive bibliography of milk pricing including articles, books, government regulations, and court cases is presented. Reference also is made to several other bibliographies on this subject. A. W. Rudnick, Jr.

674. Pupil response to experimental pricing of milk. H. L. Cook and H. W. Halvorson. Wis. Agr. Expt. Sta. Res. Bull. 190. 1956.

Milk prices were reduced in Madison and Milwaukee elementary and high schools. Price reductions of 25, 50, and 75% were made in both milk served as an auxiliary meal and in school lunches. As could be expected, consumption increased in direct relationship to price reduction. It is also noted that consumption increased more in elementary schools than in high schools as price was reduced.
A. W. Rudnick, Jr.

675. Soft serve operator—your money and the short change artist. P. E. Piper, Piper's Dairyland, Paris, Ill. Ice Cream Rev., **40**, 2: 99. 1956.

The author discusses some of the techniques used by the short change artist and ways of combating the problem. W. J. Caulfield

676. Molded milk bottle case. W. O. Farrell (assignor to The Richardson Company, Lockland, Ohio). U. S. Patent 2,758,742. 9 claims. Aug. 14, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 2: 372. 1956.

A milk bottle case molded out of plastic.
R. Whitaker

677. Dairy wax composition. R. M. Bailly, L. Silver, and H. F. Hitchcox (assignors to Esso Research and Engineering Company, Del.). U. S. Patent 2,758,100. 8 claims. Aug. 7, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 1: 190. 1956.

Detailed specifications are given for an improved type of microcrystalline wax for coating paper milk bottles. R. Whitaker

678. Paper bottle spout. R. M. Hansen. U. S. Patent 2,757,830. 9 claims. Aug. 7, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 1: 124. 1956.

A pouring spout is described located on one side of the gabled top section of a Pure-pak type of paper milk bottle. R. Whitaker

679. Milk carton dispensing machine. W. Shanhouse and E. Daher (assignors to Shanner Equipment Company). U. S. Patent 2,765,-101. 39 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 90. 1956.

Paper bottles containing milk are moved one at a time to a dispensing door by a series of small trays holding 1 carton in each tray. R. Whitaker

680. Panel structure and blank for containers. C. R. Alden (assignor to Ex-Cell-O Corporation, Detroit, Mich.). U. S. Patent 2,763,-424. 10 claims. Sept. 18, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 3: 600. 1956.

A pouring spout of sanitary design is described for a Pure-Pak type of paper milk bottle. R. Whitaker

681. Handle for milk cartons. F. Young and T. W. Munger. U. S. Patent 2,763,414. 9 claims. Sept. 18, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 3: 598. 1956.

An easily attached reusable handle for holding a paper milk bottle when the contents are being poured out. R. Whitaker

FEEDS AND FEEDING

682. Cutting alfalfa early for better quality hay and silage. D. Smith. Wis. Agr. Expt. Sta. Bull. 521. 1956.

A four year trial indicates that more hay per acre can be obtained by cutting Vernal and Ranger varieties of alfalfa 3 × rather than 2 × per season. The Grimm variety yields less hay with 3 × cuttings. All three produced high yields of protein. A number of cautions concerning fertilizing and use of insecticides are given in managing the more frequently cut hay crops. A. W. Rudnick, Jr.

683. Dispensing system for applying molasses to stock feed. A. M. Talbott. U. S. Patent 2,758,566. 2 claims. Aug. 14, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 2: 327. 1956.

Molasses, stored in a tank outside the barn, is piped into a tempering tank equipped with an electric heater. The warmed product is then diluted with water and sprayed over the cows' feed after it has been placed in position for feeding. R. Whitaker

HERD MANAGEMENT

684. Valved fountain sponge udder washer. N. Cordis. U. S. Patent 2,759,210. 11 claims. Aug. 21, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 3: 512. 1956.

A device for attaching to a hose for quickly and thoroughly cleaning udders of cows. R. Whitaker

685. Cow tail holder. E. Fischer. U. S. Patent 2,757,637. 3 claims. Aug. 7, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 1: 72. 1956.

A clamp is described for preventing a cow from swishing her tail. R. Whitaker

686. Nostril attached calf and cow weaner. O. D. Blosser. U. S. Patent 2,765,774. 6 claims. Oct. 9, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 2: 260. 1956.

A description is given for a device for attaching to the nostrils of a calf to prevent nursing at the udder. It consists chiefly of pointed wires so positioned that they prick the calf if suckling is attempted. R. Whitaker

687. Hygienic milking buckets and like containers. J. T. Grieff. U. S. Patent 2,765,093. 5 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 88. 1956.

A milking pail of nearly spherical shape designed to reduce outside contamination to the minimum. R. Whitaker

688. Barn cleaner system. N. Cordis. U. S. Patent 2,765,089. 9 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 87. 1956.

A shovel designed to operate the full length of the gutter is pulled along by a tow line attached to a drum rotated by an electric motor. R. Whitaker

689. Animal operated water bowl. R. G. Ferris (assignor to Starline, Inc.). U. S. Patent 2,764,960. 5 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 55. 1956.

A flange in the water bowl is attached to the valve controlling the water flow into the bowl. R. Whitaker

690. Cartridge-powered cow dehorner. R. D. Hoffman. U. S. Patent 2,764,813. 11 claims. Oct. 2, 1956. Offic. Gaz. U. S. Pat. Office, **711**, 1: 19. 1956.

The expanding gas from a fired cartridge operates a piston to which is attached one of two blades. The horn is severed between the two blades. R. Whitaker

691. Dust cap for milking systems. A. E. Anderson. U. S. Patent 2,763,459. 2 claims. Sept. 18, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 3: 609. 1956.

An arrangement for closing and protecting the outer surface of milking machine nipples when not in use. R. Whitaker

692. Means for milking and handling the milk of farm animals. G. Duncan (assignor to Zero Manufacturing Company, Washington, Mo.). U. S. Patent 2,763,240. 7 claims. Sept. 18, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 3: 553. 1956.

A milk cooling system, mounted on wheels, which may be rolled into position outside a milking parlor during the milking period and later moved to the milk house or elsewhere for discharge of the cooled milk. R. Whitaker

693. Anti-kicking type animal restraining device. S. Allen. U. S. Patent 2,762,334. 1 claim. Sept. 11, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 2: 324. 1956.

A device is described for strapping to the hind legs of cows to prevent kicking. R. Whitaker

ICE CREAM

694. Fast freeze for production ease. C. T. Anastos. Ice Cream Rev., **40**, 2: 34. 1956.

A recently installed wind tunnel for hardening ice cream is described. The fast freeze unit consists of double deck variable speed conveyors operating in air maintained at –40° to –50° F. and circulated at the rate of 24,000 cu. ft. per min. by means of two 36″ fans.

The wind tunnel room which is 55 ft. long and 10 ft. wide provides hardening capacity of 600 to 750 gal. per hr. The tunnel will handle 80 pt. per min., 44 qt. or 22 ½-gal. per min.

The use of this new tunnel has resulted in improved quality of product, it speeds up handling of special and emergency orders, and has eliminated deformed packages. The ice cream coming out of the wind tunnel is completely and uniformly hardened and is ready for immediate delivery and sale. W. J. Caulfield

695. Hawaii tropical fruit flavors in ice ice creams, sherbets and ices. T. N. Shaw, S. Sakata, F. P. Boyle, and G. O. Sherman. Hawaii Agr. Expt. Sta. Circ. 49. 1955.

Ice cream, sherbert, and ices were made using a number of native Hawaii fruits for flavorings. Excellent ice creams were manufactured using avocado, coconut, banana, mango, lychee, and soursop for flavoring. Soursop, lychee, passion fruit, strawberry guava, common guava, mango and banana were excellent for sherberts and ices. The chemical composition of some of the fruits is given. A. W. Rudnick, Jr.

696. Ice cream freezer and dispenser. O. A. Vasby. U. S. Patent 2,758,823. 3 claims. Aug. 14, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 2: 393. 1956.

A horizontal ice cream freezer is described having an auger type agitator. R. Whitaker

697. Confection package. J. Young and B. W. Colman. U. S. Patent 2,759,831. 4 claims. Aug. 21, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 3: 667. 1956.

An insulated package for frozen novelties on a stick. R. Whitaker

698. Automatic feed for ice cream freezers. H. F. Oltz (assignor to Ar-Tik Systems, Inc., Miami, Fla.). U. S. Patent 2,760,344. 7 claims. Aug. 28, 1956. Offic. Gaz. U. S. Pat. Office, **709**, 4: 796. 1956.

An electrically energized device for introducing unfrozen mix into a freezer in proportion to the amount of frozen product dispensed from the freezer. R. Whitaker

699. Ice cream disher. A. Gordon (assignor to Ekco Products Company, Chicago, Ill.). U. S. Patent 2,761,399. 5 claims. Sept. 4, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 1: 72. 1956.

An ice cream disher is described for forming spherical shaped individual portions of ice cream. R. Whitaker

700. Marbling attachment unit for batch-type ice-cream freezer. M. R. Prouty. U. S. Patent 2,762,318. 8 claims. Sept. 11, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 2: 320. 1956.

The outlet of a freezer is modified to permit the attachment of a device for injecting pureed fruit, flavored syrup, etc. into the stream of ice cream leaving the freezer. The material is conducted from a supply tank through tubes terminating at the gate valve, so that the operation of the gate controls the flow of both ice cream and the injected material. R. Whitaker

MILK AND CREAM

701. Valve for pressurized containers. N. E. Spiess, Jr. and H. L. Henze (assignors to National Dairy Research Laboratories, Inc., Oakdale, N. Y.). U. S. Patent 2,761,593. 10 claims. Sept. 4, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 1: 121. 1956.

A rubber valve is described for dispensing whipped cream and similar products from a gas packed container. R. Whitaker

702. Process of treating and canning a beverage. L. D. Stewart (assignor to North American Milk Industries, Inc.). U. S. Patent 2,761,780. 7 claims. Sept. 4, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 1: 170. 1956.

A process is described for producing a date flavored milk beverage, consisting of flavoring homogenized milk with a date concentrate, filtering, stabilizing the filtrate, heating to about 130° F., deaerating at about 100° F. under 25

in. of vacuum, heating to about 195° F. and aseptically filling into sterilized cans, sealing under vacuum and cooling with brisk agitation.
R. Whitaker

MILK SECRETION

703. Does prepartum milking pay? R. A. Ackerman, W. Va. Univ. Hoard's Dairyman, **101**, 20: 1011. 1956.

One hundred cows milked for two weeks prepartum were compared with a similar number as controls. Holsteins and Ayrshires produced larger amounts of milk before freshening than Jerseys.

No differences in milk yields were noted in the lactation totals of the cows in the two groups. The incidence of milk fever, the length of the gestation period and the birth weights of the calves were not affected by prepartum milking. Prepartum milking increased the number of cases of retained placenta and more congestion of udders was noted. The composition of the milk was normal by calving time, if the cows were producing up to 20 lb. daily. The growth of the calves was not affected.

The author decided that prepartum milking had no advantages. A. R. Porter

NUTRITIVE VALUE OF DAIRY PRODUCTS

704. The evolution of infant feeding and the advantages and disadvantages of soured milk mixtures. W. B. Blue and W. C. Davison, Duke Univ. School of Med., Durham, N. C. Postgraduate Medicine, **20**, 1: 41. 1956.

A formula prepared from evaporated milk acidified with lactic acid has been used at the Duke Hospital for twenty-five years. Infants thrive on the lactic acid milk as well as they do on breast milk. The advantages of the formula are discussed in detail. The authors describe the history of infant feeding and the various theories underlying the procedures commonly accepted at the various periods. The authors' observations in visiting childrens hospitals throughout the world are reported, and some comments on the inadequacy of teaching infant feeding in the medical schools are presented.
F. E. Rice

705. Emotional aspects of feeding the aged. W. Blumenthal, Jewish Home for the Aged, Chicago. J. Am. Dietetic Assoc., **32**, 9: 829. 1956.

Under the head of "Symbolism in food" the author proposes that milk may be accepted or rejected depending on reactions other than whether the person likes or dislikes the taste of milk. If the feeling of security and comfort associated with his earliest experiences with milk is stronger, milk is likely to be accepted. If the subconscious desire to be independent is stronger, the older person may reject milk even if he may really like it. F. E. Rice

706. Growth-promoting action of dietary fat. Editorial-Review. Nutrition Rev., **14**, 10: 305. 1956.

Rats were fed diets varying from 10 to 73 calories-% using 20 different fats. Inhibition in growth occurred with high levels of most fats. One of the exceptions was butter fat, which caused no significant inhibition in growth as the % of fat in the diet was increased. Various aspects of the subject are reviewed with references, including the possibility of toxic components in some of the fats, for example, erucic acid. F. E. Rice

PHYSIOLOGY AND ENDOCRINOLOGY

707. The effect of repeated glucose injections upon milk and milk fat production. A. C. Chung and J. C. Shaw, Md. Agr. Expt. Sta., College Park. Guernsey Breeders' J., **98**, 4: 138. 1956.

Fourteen cows were divided into a control group of 6 cows and two injection groups of 4 cows each. One group was injected with 250 ml. of 50% glucose daily for 6 consecutive days. The other group was injected at the same rate for 2 days followed by 500 ml. of 50% glucose daily for the next 4 days. Total milk and butterfat production was determined for 6 days preliminary to the injection period, during the injection period and for 6 days post-injection.

No appreciable difference in milk or milk fat production resulted from the injections. Repeated injections of glucose have no stimulatory or inhibitory effects on milk or butterfat production of normal cows on adequate rations.
A. R. Porter

708. Osmotic pressure in the intestine of the cow. E. Brouwer and E. J. V. Weerden. Nature, **178**, 4526: 211. 1956.

The osmotic pressure in most of the intestine of the cow is slightly hypotonic with regard to the blood. The osmotic pressure was determined from the freezing point of the juice expressed from the faeces. It ranged from 0.24° to 0.43° C. with a mean of 0.35° C. For the blood the value 0.56° to 0.59° C. was obtained. In the small intestine strong hypotony occurs.
R. Whitaker

709. Metabolism of glycerol, sorbitol and related compounds by spermatozoa. T. Mann and I. G. White. Nature, **178**, 4525: 143. 1956.

In these experiments washed raw spermatozoa were mixed with the compounds studied. Glycerol was utilized aerobically, the oxygen

uptake increased with time, and lactic acid was produced. The production of lactic acid was influenced by the amount of phosphate present. The reactions mentioned depended on the viability of the spermatozoa. Of several alcohols tested, only sorbitol increased the oxygen uptake and lactic acid content. R. Whitaker

SANITATION AND CLEANSING

710. House fly control in dairy barns, 1955; M. A. Price, Texas Agr. Expt. Sta. Prog. Rpt. 1867. 1956.

In dairy barns the following concentrations of insecticides gave protection from houseflies for the number of days noted: Malathion (emulsifiable concentrate), 2.5% protection for 47 d., 5.0%, 87 d.; Diazinon (wettable powder) 0.5%, 87 d., 1.0%, 73 d.; (emulsifiable concentrate) 0.5%, 74 d., 1.0%, 48 d.; Chlortion (emulsifiable concentrate) 0.5%, 20 d. The 1.0% Diazinon was used in barns with poor sanitation and numerous nearby flybreeding areas. A. W. Rudnick, Jr.

711. Device for cleaning milk conduits of machine milking plants. E. Redin (assignor to Aktiebolaget Manus, Norrkoping, Sweden). U. S. Patent 2,763,017. 1 claim. Sept. 18, 1956. Offic. Gaz. U. S. Pat. Office, **710**, 3: 498. 1956.

A device for cleaning the interior walls of milking machine hose lines. It consists of a cylindrical core with a series of flexible flanges, evenly spaced, and of such diameter thickness and placement that the walls of the tube are cleansed when the device is moved through it when straight or bent. R. Whitaker

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